Ethylene oxide-propylene oxide copolymers in yeast fermentations

Thesis

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ETHYLENE OXIDE–PROPYLENE OXIDE COPOLYMERS

IN YEAST FERMENTATIONS

A thesis submitted to the Open University for the degree of Master of Philosophy.

by

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ABSTRACT

Ethylene oxide-propylene oxide copolymers are prepared by addition of ethylene oxide to polypropylene glycol. One of the uses of these compounds is to inhibit foam formation in aqueous media.

The main purpose of this work has been to try to determine how variation of molecular weight and ethylene oxide content of these polymers changes their efficiency of foam inhibition in a particular foaming system, namely an aerobic yeast fermentation. In addition attempts have been made to study the effect of the polymers upon yeast growth and again to relate this to variations in molecular weight and ethylene oxide content.

The results obtained show that polymers with ethylene oxide contents below 15% by weight were most effective with respect to inhibition of foaming. Increasing the molecular weight of these polymers resulted in more effective control of foaming.

The results also show that all of the polymers studied caused a reduction in yeast growth although this effect was less marked with a polymer having a molecular weight of 2271 and an ethylene oxide content of 11% by weight.

Methods have also been developed for purification of the commercially available polymers and characterisation of them using gel permeation chromatography and carbon-13 NMR.

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PART A

CHARACTERISATION OF ETHYLENE OXIDE-PROPYLENE OXIDE COPOLYMERS

CHAPTER 1

1. INTRODUCTION

Ethylene oxide-propylene oxide block copolymers are prepared by the addition of ethylene oxide to poly (propylene glycol) in the presence of an alkaline earth metal catalyst at temperatures of 130-190°C (1-5). The reaction can be represented as follows:

$$HO(C_3H_6O)_xH + (y+z)\ \underset{\diagdown O \diagup}{CH_2-CH_2} \rightarrow H\text{-}(\text{-}O\text{-}CH_2\text{-}CH_2)_y\text{-}(\text{-}O\text{-}\underset{\displaystyle CH_3}{\underset{|}{CH}}\text{-}CH_2)_x\text{-}(\text{-}O\text{-}CH_2\text{-}CH_2)_zOH$$

A range of these polymers is commercially available from Pechiney Ugine Kuhlman Ltd. under the trade name Pluronic (6). It is possible to vary the values of x, y and z in order to modify both the molecular weight and/or the hydrophobicity of the polymer. The physical form of the polymers at room temperature varies with molecular weight and degree of ethoxylation. Those polymers containing higher proportions of ethylene oxide tend to be solids.

The commercially available materials are designated with a particular code. This code consists of a letter followed by a two or three digit number. The letter indicates the physical form of the polymer at room temperature e.g. L (liquid), P (paste), F (flake). The number gives an indication of both molecular weight and degree of ethoxylation. The first one or two digits indicates the typical molecular weight of the poly (propylene glycol) hydrophobic base prior to ethoxylation e.g.

First Digit	Typical Molecular Weight of Hydrophobic Poly (Propylene Glycol) Base
3	950
4	1200
6	1750
7	2050
8	2250
9	2750
10	3250

The second or third digit indicates the percentage of polyoxyethylene in the total molecule, e.g. second or third digit 1 - equivalent to 10-19% polyoxyethylene, second digit 6 - equivalent to 60-69% polyoxyethylene.

These polymers have a variety of commercial applications, e.g. as emulsifiers, lubricants, foam control agents (7). As a result of this the commercially available materials are often of fairly broad composition with respect to molecular weight and degree of ethoxylation. In order to obtain information on the samples of Pluronics obtained, attempts were made to characterise the polymers with respect to molecular weight and degree of ethoxylation.

The degree of ethoxylation was determined from data obtained by both ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. The molecular weight distributions were determined by gel permeation chromatography and expressed as M_n and M_w where M_n is the number average molecular weight and M_w is the weight average molecular weight. M_n is defined as the total weight w of all the molecules in a polymer sample divided by the total number of moles present. The number average molecular weight is defined by:-

$$M_n = \frac{w}{\sum N_x} = \frac{\sum N_x M_x}{\sum N_x}$$

where the summations are over all the different sizes of polymer molecules from x = 1 to x = ∞ and Nx is the number of moles whose weight is M_x. M_n is determined from colligative properties, whereas M_w is obtained from light scattering measurements and is defined as:

$$M_w = \frac{\Sigma N_x M_x^2}{\Sigma N_x M_x}$$

Initial results yielded information on the degree of ethoxylation but showed the polymers to have a wide molecular weight distribution. As a result of this attempts were made to purify the polymer by removing much of the high molecular weight "tail" present in many of the samples. These attempts appear to have been successful and it was felt that the polymers were well characterised and suitable for evaluation in the fermentation work.

CHAPTER 2

2. RESULTS AND DISCUSSION

2.1 Preparation of Samples

The following samples were received from Pechiney Ugine Kuhlman and were used as the basis for the work: Pluronics L31, L61, L62, L63, L64, F68, L81, L92, L101 and L121.

Both GPC (for molecular weight distribution) and NMR (for degree of ethoxylation) indicated that the samples were, as anticipated, ill defined with GPC data indicating broad molecular weight distributions (see Section 2.3).

For later fermentation work it was considered necessary to have more closely characterised polymers, so that the effect of varying both molecular weight and degree of ethoxylation of the polymers could be related to fermentation parameters such as foam volume, yeast yield, pH and pO_2. Hence a method of "purifying" the polymers was required. The idea of fractional precipitation of the polymers was favoured. A "salting out" technique (8) is often used in the purification of ethoxylated materials. This technique was used as the basis for the development of a method applicable to ethylene oxide-propylene oxide copolymers (see Section 3.1). The technique relies on the increasing insolubility of these polymers in aqueous solutions of increasing ionic strength.

Propylene oxide homopolymers with a molecular weight above 800-900 become essentially water-insoluble and their insolubility increases with increasing molecular weight. However incorporation of hydrophilic polyoxyethylene groups increases their water solubility. The higher the degree of ethoxylation, the more water-soluble the material becomes.

Hence it was anticipated that the salting out technique would remove any propylene oxide homopolymers present and also remove any higher molecular weight copolymers.

Both GPC (see Section 2.4) and NMR (see Section 2.3) confirm this but would suggest that the concentration of propylene oxide homopolymers in the original samples was small and may also indicate that ethylene oxide homopolymers were present in the original samples.

Problems arose with the salting out technique, in that the original polymers yielded many fractions as shown below (tables 2.1.1. and 2.1.2.). This was due to the original polymers having a wide distribution of fractions that had differing hydrophobicities and as such had differing solubilities in solutions of increasing ionic strength.

TABLE 2.1.1

	Weight Percentage Of Original Polymer Recovered				
Fraction	Pluronic L31	Pluronic L61	Pluronic L62	Pluronic L63	Pluronic L64
1	0.15	3.39	3.24	4.32	2.90
2	1.48	5.42	2.36	3.58	0.82
3	2.88	5.44	3.10	3.68	1.16
4	8.53	16.67	1.98	67.80	77.20
5	1.20	24.94	74.60	< 0.1	< 0.1
6	69.25	13.31	< 0.1	< 0.1	< 0.1
7	< 0.1	8.88	< 0.1	< 0.1	< 0.1

TABLE 2.1.2

Fraction	Weight Percentage of Original Polymer Recovered				
	Pluronic F68	Pluronic L81	Pluronic L92	Pluronic L101	Pluronic L121
1	4.78	0.30	2.24	1.34	1.08
2	4.14	1.63	1.56	2.26	1.14
3	5.45	1.33	3.82	79.80	3.72
4	58.05	13.62	5.08	6.98	69.94
5	< 0.1	52.19	7.28	< 0.1	7.78
6	< 0.1	17.14	62.54	< 0.1	< 0.1
7	< 0.1	4.94	5.32	< 0.1	< 0.1

In addition to the above fractions, a large number of others (each representing < 0.1% by weight of the original polymer) were recovered in each case. Despite the polymers, with the exception of the highly hydrophilic Pluronic F68, responding to the salting out technique, it was felt that too great a number of fractions had been recovered to be useful in later work. Hence the salting out technique was modified (see Section 3.1) for each polymer in as much as larger aliquots of 25% w/v aqueous sodium chloride were added to obtain larger weight percentage fractions yet still retaining the initial aims i.e. removal of homopolymers and high molecular weight copolymers. This resulted in the following samples being obtained (Table 2.1.3).

TABLE 2.1.3

Polymer (Pluronic)	Fraction Weight (%) Of Recovered Polymer	Fraction Weight (%) Of Initial Polymer Weight
L31	94.60	78.98
L61	80.00	62.44
L62	93.43	79.68
L63	90.05	71.48
L64	94.05	77.20
F68	87.67	63.50
L81	91.00	82.85
L92	91.33	80.22
L101	96.02	86.78
L121	92.90	77.72

As shown in Section 2.3 and Section 2.4 these samples did appear to be better defined and the salting out technique appears successful for all of the original polymers, with the exception of Pluronic F68. Even after purification this polymer showed a broad molecular weight distribution. However as this particular polymer was considered unlikely to be of value in the fermentation stage of the work, no further attempts at purification were attempted.

The remaining purified samples were given the suffix P for later stages of the work, e.g. the purified polymer fractionated from Pluronic L31 was referred to as Pluronic L31P.

2.2 Other Methods

Although it was anticipated that NMR and GPC would be the most useful tools for the characterisation of the polymers, a number of other methods were examined with a view to elucidating information on the polymers. These are outlined below.

2.2.1 Hydroxyl Value

Hydroxyl value was used to characterise the polymers. The procedure relies on the reaction of a known weight of anhydride with the hydroxyl groups of the polymer to form the corresponding ester. The amount of anhydride consumed is determined by hydrolysing the excess anhydride and titrating the resulting acid with potassium hydroxide. The difference between this titration and a blank, where all of the anhydride is hydrolysed to the acid, gives the number of hydroxyl groups present in the polymer. The hydroxyl value is defined as the number of milligrams of potassium hydroxide equivalent to this value. Once the hydroxyl value is known, as the Pluronics have two hydroxyl groups per molecule, the number average molecular weight can be calculated.

It was suspected that the cited molecular weights for the Pluronics (6) were determined from the hydroxyl value. The method of Sully (9), see Section 3.2, was used to confirm this. The results below show good correlation with literature values.

TABLE 2.2.1

Pluronic	Molecular Weight (Literature Value) (6)	Molecular Weight From Hydroxyl Value
L31	1100	1125
L61	2000	2135
L62	2500	2455
L63	2650	2620
L64	2900	2840
F68	8350	7525
L81	2750	2790
L92	3650	3240
L101	3800	3765
L121	4400	4300

Obviously these data shed no light on molecular weight distribution and they also give ambiguous results in the presence of low molecular weight hydroxyl containing compounds. Hence the method was abandoned.

2.2.2 Infra-red Spectroscopy

G.F. Longman (8) describes two overtone methods for determining ethylene oxide content in ethylene oxide-propylene oxide copolymers. One relies on the ratio of intensities of peaks due to methyl and methylene groups, whereas the other relies on differing intensities of absorption of polyethylene and polypropylene glycols. This latter method can be prone to difficulties due to water contamination.

Spectra of the various Pluronics show differing absorption patterns depending upon the polyoxyethylene content of the polymers. However the differences are small and do not appear quantitative.

Methods employing near infra-red spectroscopy (10) are also available and suggest good reliability for determination of ethylene oxide content of ethoxylated polymers. Such techniques are also not affected by the presence of water in the polymer. Unfortunately suitable equipment is not available to the author for him to verify this.

2.2.3 Pyrolysis

Controlled pyrolysis of the polymers has been claimed to liberate stoichiometric quantities of acetaldehyde and propionaldehyde which can be determined colourimetrically (11). However pyrolysis under a variety of conditions failed to give reproducible results and yielded little information on ethylene oxide or propylene oxide content of the polymers.

2.2.4 Vapour Pressure Osmometry (V.P.O.)

Conventional vapour pressure osmometry was employed to evaluate a number of Pluronics (see Section 3.2). Again, however, the technique tended to be susceptible to contamination by low molecular weight materials in the Pluronics. None of the polymers evaluated showed sufficient linearity of response with respect to molecular weight and vapour pressure, and this suggests V.P.O. is not a suitable method for determining molecular weight of these polymers, in some agreement with previous work (12).

All of the above methods were abandoned in favour of NMR and GPC.

2.3 Nuclear Magnetic Resonance Spectroscopy (N.M.R.)

A number of methods (13-18) have been described employing NMR for the characterisation of ethoxylated materials. Initially a simple ^{1}H NMR method was used for all of the original Pluronic samples. NMR methods for ethylene oxide-propylene oxide copolymers are based on characteristic signals for protons in the methyl group derived from propylene oxide and for protons attached to other carbon atoms. Details of the method used are shown in Section 3.3. The use of ^{13}C NMR was thought to offer advantages over simple proton NMR, since the expanded chemical shift range was thought likely to yield a more accurate picture of ethylene oxide content. Again the method employed is detailed in Section 3.3.

Typical spectra for both ^{1}H and ^{13}C NMR of the Pluronics are shown in Appendix 99. They are described below:-

TABLE 2.3.1 Typical chemical shifts for ^{1}H NMR of Pluronics

Chemical Shift δ/ppm	Multiplicity	Assignment
1.05-1.20	Multiplet	(H_a) Methyl protons from propylene oxide
3.40-3.50	Broad Multiplet	(H_b) - CH_2 - CH_2 - from ethylene oxide (H_c) - CH - CH_2 - from propylene oxide (H_d) - terminal OH

The broad band centred at δ 3.40-3.50 would also contain a small contribution from the terminal hydroxyl protons as shown below:

$$H_{(d)}\text{-}(O\text{-}CH_{2(b)}\text{-}CH_{2(b)})_y\text{-}(O\text{-}\underset{\underset{CH_{3(a)}}{|}}{CH}_{(c)}\text{-}CH_{2(c)})_x\text{-}(\text{-}O\text{-}CH_{2(b)}\text{-}CH_2)_{(b)z}\text{-}OH_{(d)}$$

Hence this relatively simple spectrum affords a way of determining the percentage of ethylene oxide in the polymers directly from the integration ratio of the spectrum.

The percentage of ethylene oxide is determined as follows:-

The ratio of propylene oxide (PO) to ethylene oxide (EO) by weight is given by the following integrations:-

$$\frac{PO}{EO} = \frac{\int CH_{3(a)}/3}{\left[\int(CH_2 - CH_2 -)_{(b)} + \int(CH_2 - CH)_{(c)} - \int(CH_3-)_{(a)}\right]/4} \times \frac{\text{Molar mass PO}}{\text{Molar mass EO}}$$

Where $\int$ is the integration value attributed to the particular signal.

Also EO + PO = 1, and

$$\text{hence weight percent of EO} = \frac{1}{1 + PO/EO} \times 100$$

This method was applied to all of the polymers evaluated with the exception of Pluronic F68. The ^1H spectrum of this polymer was unusual in showing no signal at δ 1.05-1.20. This anomaly may be due to a very low concentration of propylene oxide in the polymer. With the exception of Pluronic F68, the spectra compared well with typical spectra for copolymers of ethylene oxide with propylene oxide (14).

The ^{13}C NMR spectra were of better resolution than the proton spectra and allowed better assignment, mainly due to the expanded chemical shift range as shown below:-

TABLE 2.3.2 Typical chemical shifts for ^{13}C NMR of Pluronics

Chemical Shift (δ/ppm)	Assignment
17.7	Methyl carbon in propylene oxide
65.9	Methylene carbon in ethylene oxide
69.0	Methylene carbon in propylene oxide
70-71	Methylene carbon in ethylene oxide
73-74	Methylene carbon in propylene oxide
75-76	Methine carbon in propylene oxide

Again integration of the spectra readily yielded information on the weight percent of ethylene oxide in the polymers.

$$\frac{PO}{EO} = \frac{(\int 17.7 + \int 69.0 + \int 73\text{-}74 + \int 75\text{-}6)/3}{(\int 65.9 + \int 70\text{-}71)/2} \times \frac{\text{Molar Mass PO}}{\text{Molar Mass EO}}$$

Where $\int$ is the integration value attributed to the particular signal.

$$\text{Again Weight percent of EO} = \frac{1}{1 + PO/EO} \times 100$$

All of the ^{13}C spectra were of a similar type.

Using these methods the following results were obtained for the weight percentage of ethylene oxide in the original polymers.

TABLE 2.3.3 Weight percentage of ethylene oxide in Pluronic samples

Sample	Weight Percentage EO In Original Pluronic		Literature Value (16)
	^{1}H	^{13}C	
Pluronic L31	24.9	16.6	10-19
Pluronic L61	15.0	12.5	10-19
Pluronic L62	32.0	27.0	20-29
Pluronic L63	36.6	27.2	30-39
Pluronic L64	43.7	42.5	40-49
Pluronic L81	23.1	13.5	10-19
Pluronic L92	30.3	22.1	20-29
Pluronic L101	15.0	12.0	10-19
Pluronic L121	21.6	12.7	10-19

Generally correlation between the two methods is poor, with ^{1}H NMR giving values higher than ^{13}C NMR. ^{1}H NMR also gave values higher than literature figures. With the exception of Pluronic L63 the values obtained from ^{13}C NMR fell within the literature range of figures.

As outlined in Section 2.1 the polymer samples were re-examined after fractionation. There were no significant changes in the chemical shifts for either ^{1}H or ^{13}C NMR spectra. However, treatment of the results gave the following values of the weight percentage of EO in the purified samples.

TABLE 2.3.5 Weight percentage of ethylene oxide in purified Pluronic samples

Sample	Ethylene Oxide Contents (%) In Purified Pluronic	
	^{1}H	^{13}C
Pluronic L31P	15.4	10.9
Pluronic L61P	14.7	11.0
Pluronic L62P	27.6	23.5
Pluronic L63P	31.1	26.1
Pluronic L64P	40.0	38.9
Pluronic L81P	21.1	14.6
Pluronic L92P	28.4	21.2
Pluronic L101P	15.5	12.9
Pluronic L121P	18.0	12.6

The purification of the polymers appeared to have resulted in a reduction in ethylene oxide content (with the exception of Pluronic L101P where little change had occurred). Again the values obtained from ^{13}C spectra were lower than the values obtained using ^{1}H spectra. The reduction in ethylene oxide content was due to removal of ethylene oxide homopolymers which remained in the aqueous phase during the salting out procedure, and also due to the loss of high molecular weight copolymers containing high levels of ethylene oxide.

The use of ^{1}H and ^{13}C NMR yielded direct information on the composition of the polymers with respect to ethylene oxide content. Obviously the discrepancy between the ^{1}H and ^{13}C figures was perturbing and required clarification. A number of ethylene oxide and propylene oxide homopolymers were examined, both singly and as mixtures. The results of these evaluations are shown below. The mixtures were prepared to give theoretical ethylene oxide contents from 5% to 25% by weight of mixture.

TABLE 2.3.5 Weight percentage of ethylene oxide in mixtures of homopolymers

Ethylene Oxide Content (%) As Prepared	Ethylene Oxide Content (%) From Spectra	
	^{1}H	^{13}C
0	0	0
5	11	5.6
10	17	10.4
15	24	16.7
19.5	28	17.2
25	31	25.0

Obviously the discrepancy between the methods was further highlighted. The data from the ^{13}C spectra agreed well with the known values for the simple mixtures and was used as the basis of further work.

The reason for the high values obtained from the ^{1}H spectra is not known. However, it is suspected that water contamination of the polymers may have led to the spurious results. Although all of the samples were dried before analysis, ethylene oxide containing polymers are notoriously hygroscopic and further contamination with water cannot be ruled out. If this did occur signals from these protons may well have been hidden under the signal attributed solely to the CH_2 and CH protons.

2.4 Gel Permeation Chromatography (G.P.C.)

Gel permeation chromatography has long been used as a reliable technique for determining the molecular weight distribution of polymer systems (19). A number of systems have been developed for examining ethylene oxide homopolymers (19, 20) and latterly copolymers of ethylene oxide and propylene oxide (21-23).

These techniques have been used for examining the Pluronics in this work. Experimental details are outlined in Section 3.4. All of the polymer samples were examined using ethylene oxide homopolymers as calibration standards.

Initially the original Pluronics were examined, and a typical chromatogram and computer printout giving details of number average (M_n) and weight average (M_w) molecular weight are attached in appendix 100.

The results for the samples examined are shown below in Table 2.4.1.

TABLE 2.4.1 Molecular weight of Pluronics from G.P.C. data

SAMPLE	M_n	M_w
Pluronic L31	1357	1445
Pluronic L61	2256	2461
Pluronic L62	2640	3068
Pluronic L63	2740	3155
Pluronic L64	3167	3467
Pluronic F68	5536	7102
Pluronic L81	3111	3427
Pluronic L92	3764	4312
Pluronic L101	3897	5011
Pluronic L121	4152	5636

These results show poor agreement with literature figures and with the results obtained using the hydroxyl values, possibly indicating the sensitivity of the latter technique to the presence of low molecular weight hydroxyl containing compounds.

The chromatograms of the Pluronics were all of a broadly similar type showing one distinct but broad peak. In some of the higher molecular weight polymers there was also a small, but significant (5-10% by weight) lower molecular weight peak. Generally the chromatograms were not considered satisfactory, showing the presence of much high molecular weight material in the peak "tail". This was considered to be copolymer material.

The low molecular weight material, which is likely to be ethylene oxide homopolymers, was also considered undesirable for the purpose of this work. It was anticipated that the fractionation technique described in Section 2.1 would result in removal of the high molecular weight "tail" and would also leave the low molecular weight (M_w < 1,000) material in the aqueous phase (see Section 3.1).

After fractionation chromatograms on the purified polymers were prepared and molecular weight values are shown below.

TABLE 2.4.2 Molecular weight of purified Pluronics from G.P.C. data

Sample	M_n	M_w
L31P	690	974
L61P	1945	2271
L62P	1901	2363
L63P	1835	2273
L64P	1695	2477
F68P	1228	4080
L81P	2392	2814
L92P	2814	3427
L101P	2983	3893
L121P	2944	4027

These results, in combination with the chromatograms, show that much of the high molecular weight material had been removed resulting in a fall in the molecular weight of all of the samples examined. In all cases the low molecular weight fraction present in the original samples had been removed. On the basis of the GPC results all of the polymers, with the exception of Pluronic F68, appeared to have responded well to the fractionation technique resulting in chromatograms showing one well defined peak. Pluronic F68 did not respond to the technique. The original polymer had a chromatogram showing a number of broad ill defined peaks. After fractionation the chromatogram was still ill defined showing a number of peaks. It is thought that the highly hydrophilic character of this polymer had rendered it less likely to partition into the non-aqueous phase during the fractionation, and as a result none of the high molecular weight hydrophilic fractions were removed.

2.5 Summary

The development of a fractionation technique dependent upon differences in hydrophilic character and determination of both molecular weight and ethylene oxide content of the polymers by the use of GPC and ^{13}C NMR spectroscopy, has enabled purification and characterisation of the polymers to be carried out.

As a result of this, nine "purified" characterised polymers were suitable for use in other stages of the work.

The methods used do not appear suitable for use with high molecular weight polymers containing high levels of ethylene oxide such as Pluronic F68. No further attempts were made to characterise this particular polymer as its highly hydrophilic character suggests it would be of little value in the fermentation stage of the work.

CHAPTER 3

3. EXPERIMENTAL

3.1 Sample Preparation

Reagents and Materials

1. Methanol (AnalaR); dried over magnesium
2. 25% w/v aqueous sodium chloride (AnalaR)
3. Toluene (AnalaR)
4. Filter paper; Whatman No. 42

Method

Polymer (5 g) was dissolved in methanol (45 cm^3). To this solution sodium chloride solution (10 cm^3) was added. The mixture was then shaken for 30 seconds and allowed to stand. A white emulsion was produced (see note 3.1.1.) and the mixture was left to stand until a polymer rich layer formed at the surface (see note 3.1.2.). The polymer rich layer was then carefully removed and evaporated at 105°C for 2 hours. After cooling, toluene (10 cm^3) was added and the mixture swirled until the polymer dissolved. The mixture was then filtered to remove any residual sodium chloride. The sodium chloride was washed with further toluene (10 cm^3). The filtrates were combined and evaporated at 130°C for 1 hour. The resultant polymer was then cooled and weighed. The whole procedure was then repeated with further additions of sodium chloride solution (10 cm^3 aliquots) until subsequent additions of sodium chloride solution did not result in emulsion formation.

3.1.1.

The more hydrophilic polymers formed emulsions less readily and addition of further aliquots (10 cm^3) of sodium chloride solution was required.

The fractions shown in Table 2.1.3 were obtained by adding a number of aliquots of sodium chloride solution (shown under A in table 3.1.1.1) allowing the emulsion to separate and then discarding it. Further aliquots were then added (shown under B in table 3.1.1.1), the emulsion was separated and the polymer was recovered.

Table 3.1.1.1. Volumes of sodium chloride solution added to fractionate polymers

PLURONIC	A VOLUME (cm^3) OF SODIUM CHLORIDE	B VOLUME (cm^3) OF SODIUM CHLORIDE
L31	30	50
L61	20	50
L62	20	40
L63	20	40
L64	30	10
F68	30	30
L81	30	30
L92	30	60
L101	20	30
L121	30	20

3.1.2.

The polymer rich layer took up to 24 hours to separate. This period was reduced by centrifuging the mixture for 10 minutes at 4,000 rpm.

3.2 Other Methods

3.2.1 Hydroxyl Value

Reagents and Materials

1. Xylene (GPR)
2. Stearic Anhydride (GPR); minimum assay 97%
3. Anhydrous Pyridine (GPR); freshly distilled
4. 1M Sodium Hydroxide

A sample weight of polymer equivalent to 50-100 mg hydroxyl groups, by weight of polymer, was added to xylene (15 cm^3). To this, stearic anhydride (8 g accurately weighed) was added and the mixture refluxed for 30 minutes and then cooled. Pyridine (40 cm^3) and water (4 cm^3) were added and the mixture boiled under reflux for 30 minutes, after which it was titrated hot against sodium hydroxide solution using phenolphthalein as indicator. The same procedure was carried out for a blank sample (i.e. containing no polymer).

$$\text{Hydroxyl value} = \frac{V \times 56.11}{W} \quad \text{mg KOH } g^{-1}$$

where V = (Blank - sample) titration

W = Weight of sample

and 56.11 is the molecular weight of potassium hydroxide

$$\text{Hence } M_n \text{ of Pluronic} = \frac{56110 \times 2}{\text{Hydroxyl value}}$$

where the number of hydroxyl groups per molecule is 2 and 56110 is the number of milligrams of potassium hydroxide per mole.

3.2.2. Infra-red Spectroscopy

Spectra were obtained using 2% w/v solutions of the polymers, in dry chloroform, on a Perkin Elmer 157G Grating infra-red spectrometer.

3.2.3. Pyrolysis

Reagents and Materials

1. Phosphoric Acid, 85% (Analar)
2. 0.134M Methanolic Sodium Nitroprusside
3. 0.5M Diethanolamine
4. Pyridine (GPR)

Dried polymer (1 g) was added to phosphoric acid (25 cm^3). 1 cm^3 of this mixture was then pipetted into a pyrolysis tube and heated to 300°C for 40 minutes. The liberated acetaldehyde and propionaldehyde were continuously collected in a mixture of sodium nitroprusside (4 cm^3) and diethanolamine (6 cm^3). Immediately after pyrolysis the collecting solution was cooled at 0°C for 19 minutes with stirring. Within 1 minute 0.5 cm^3 of this mixture was added to pyridine (9.5 cm^3). The colour intensity of the resulting solution was recorded at 565 nm (acetaldehyde) and 405 nm (propionaldehyde).

3.2.4. Vapour Pressure Osmometry

2 g of polymer (dried for 1 hour at 105°C) were dissolved in 8 g of dry toluene.

A Knauer vapour pressure osmometer consisting of two thermistor beads in a thermostatted cell (+/- 0.001°C) was used. The right hand-side bead was for pure solvent and the left-hand side for the test solution. The system was zeroed using pure solvent and the test solution then examined at its original concentration followed by dilutions at 50% and 25% of the original concentration.

3.3 NMR Spectroscopy

All of the spectra were produced on a Jeol 90 FXQ spectrometer (90 MHz, Fourier Transform) with the following conditions:

Sample: Dried Pluronic mixed in approximately equal volumes with C_6D_6.

Internal Standard: Tetramethylsilane

^1H Spectra: Single pulse. Peak area determined by instrumental integration

^{13}C Spectra: Multiple pulse. 2 s. delay between pulses to allow for nuclear relaxation. Signal areas determined by instrumental integration.

3.4 G.P.C.

Molecular weight distributions were investigated using the following equipment and computer analysis of chromatograms.

a) Columns

6 Styragel columns (4" x 5/16" internal diameter) with the following nominal porosities: 700-2000 Å
500-2000 Å
500 Å
200-500 Å
150-350 Å
50-80 Å

b) Metering Pump

Metering Pumps Ltd.; Series II micropump. Stroke rate 1 cm^3 $min.^{-1}$

c) Injection Valve

6 port. 2 cm^3 loop

d) Samples

Concentration: 2.5 g dm^{-3} for purified polymers (i.e. 5 mg load)
: 2.0 g dm^{-3} for original Pluronics (i.e. 4 mg load)

e) Solvent

Tetrahydrofuran : distilled and degassed

f) Detectors

R.I.: Waters Associates 401 differential refractometer

g) Recorder

Leeds and Northrop strip chart

h) Calibration

With polyethylene glycols up to molecular weight of 20,000.

PART B

FERMENTATION STUDIES

Chapter 4 - Introduction

4.1 Yeast Classification

The term yeast designates a type of fungi, which have certain common characteristics but may nevertheless belong to differing classes.

A detailed discussion of yeast classification is given by A H Cook (24), A H Rose and J S Harrison (25).

i) The Ascosporogenous Yeasts

These yeasts form ascospores in the cell which is called the ascus. These organisms belong to the family of the Saccharomycetaceae and include in their many genera the Saccharomyces. It is one of these genera which was used in the work described later.

ii) The Ballistosporogenous Yeasts

These yeasts form ballistospores which are ejaculated from the cell by a drop excretion mechanism. They belong to the family Sporobolomycetaceae. The genera within this family are characterised by the shape of the ballistospores formed.

iii) The Cryptococaceae Yeasts

These yeasts form neither ascospores nor ballistospores, being classed as the fungi imperfecti.

In general, classification of the yeasts is centred on their mode of reproduction. Yeasts tending to show no reproduction by sexual means tend to be classified in Section 4.1 (iii).

4.1.1 Saccharomyces Cerevisiae NCYC 990

This particular strain of yeast is one of a large number belonging to the genus Saccharomyces Cerevisiae. The genus is considered to be the most important of the Saccharomyces. The genus as a whole consists of yeasts that have the ability to ferment a number of sugars well. Generally the cells are round to oval and show budding. Sporulation takes place immediately after conjugation.

Strains of the genus Saccharomyces cerevisiae are widely used as bakers' yeasts, brewers' yeasts, wine yeasts etc. Specific information relating to the classification of the genus has been published by Wiles (26).

The specific strain used in this work was chosen from the National Collection of Yeast Cultures (NCYC) as being a typical example of a strongly-fermenting yeast in this genus. It shows typical ascospore formation and strongly assimilates glucose. Its cells are oval in shape and are normally paired. Cell division is by multipolar budding. Specific information relating to the culture, growth, fermentation, etc of this yeast is detailed in Appendix I.

4.2 Biochemistry and Cell Wall Physiology of Yeasts

No detailed information has been published on either the biochemistry or cell wall physiology of Saccharomyeas Cerevisiae NCYC 990. However, this particular strain is thought to be typical of the genus as a whole.

It is important to consider briefly the biochemical aspects of fermentation as it is the purpose of this work to investigate the effect of a number of ethylene oxide-propylene oxide copolymers on some fermentation parameters. Only the cell wall, rather than the whole cell, physiology will be considered as the major effect of the ethylene oxide-propylene oxide copolymer is likely to be upon the cell wall rather than the cell contents, as polymers of this molecular weight may not be readily transported through the cell wall.

4.2.1. Biochemistry of Yeast Fermentation

Fermentation of carbohydrates by yeast can take place under a number of conditions. The choice of conditions dictates the final products of the fermentation.

Three basic cases can be considered.

a) Anaerobic Conditions

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2$$

Under these conditions oxygen is excluded from the fermentation and ethanol is the major product. These conditions are typically employed in alcohol production.

b) Partial Aerobic Conditions

$$2\ C_6H_{12}O_6 + 3O_2 \longrightarrow 6(COOH)_2 + 6\ H_2O$$

Under these conditions a limited amount of oxygen is available for the growth of the yeast.

c) Completely Aerobic Conditions

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$$

It is this type of fermentation, where oxygen supply is not limited that is the concern of this work.

The thermodynamics of the three processes are markedly different, with anaerobic conditions being most energy demanding.

The details of the biochemical pathways involved in aerobic fermentation have been described by various authors. (27 - 33)

Some of the principles involved in aerobic yeast fermentations will be discussed in Section 4.3.

4.2.2 Cell Wall Physiology

As mentioned in Section 4.2 no detailed information is available concerning the cell wall physiology of Saccharomyces Cerevisiae NCYC 990, however the details given below for the genera Saccharomyces Cerevisiae are expected to be applicable to NCYC 990. A diagram of a typical resting cell of Saccharomyces Cerevisiae is shown below.

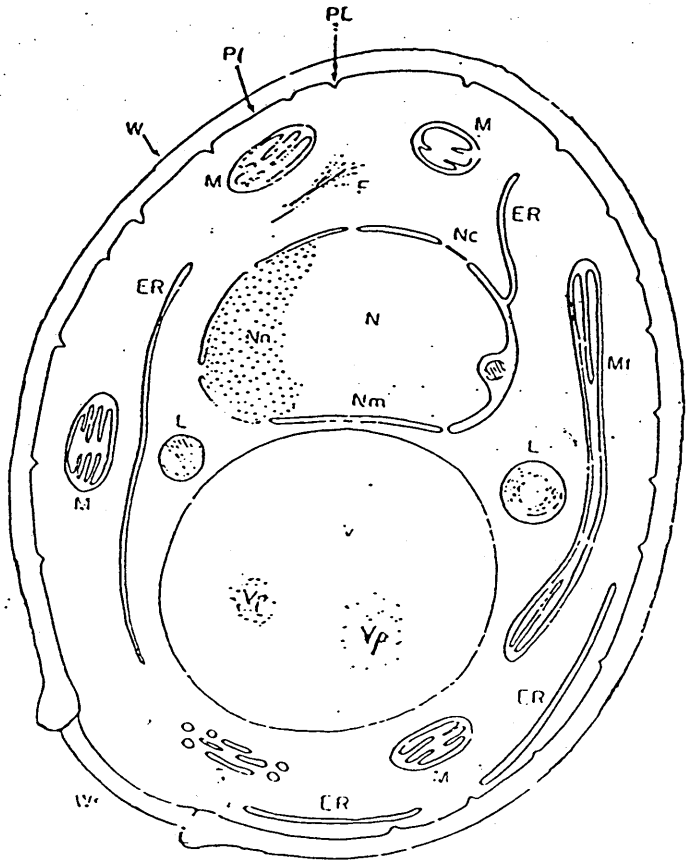


Diagram of a resting cell of baker's yeast (Saccharomyces Cerevisiae). ER indicates endoplasmic reticulum; pER, proliferating endoplasmic reticulum: ERv, vesicles derived from endoplasmic reticulum; F, filament: G, Golgi apparatus; Gl, glycogen; L, lipid granule (sphaerosome); M, mitochondrion; Mt. thread-like mitochondrion; N, nucleus; Nc, centriolar plaque; Nm, nuclear membrane; Nn, nucleolus; Ns, spindle apparatus; Pi, invagnination; Pl. plasmalemma; Pp, plasmalemma particle; R, ribosome; V, vacuole; Vp, polymetaphosphate granules; W. Cell wall (1, outer; 2, middle; 3, innermost layer); Wf, cell wall fibrils; Ws, bud scar

Also shown are the main cytological events in a budding yeast.

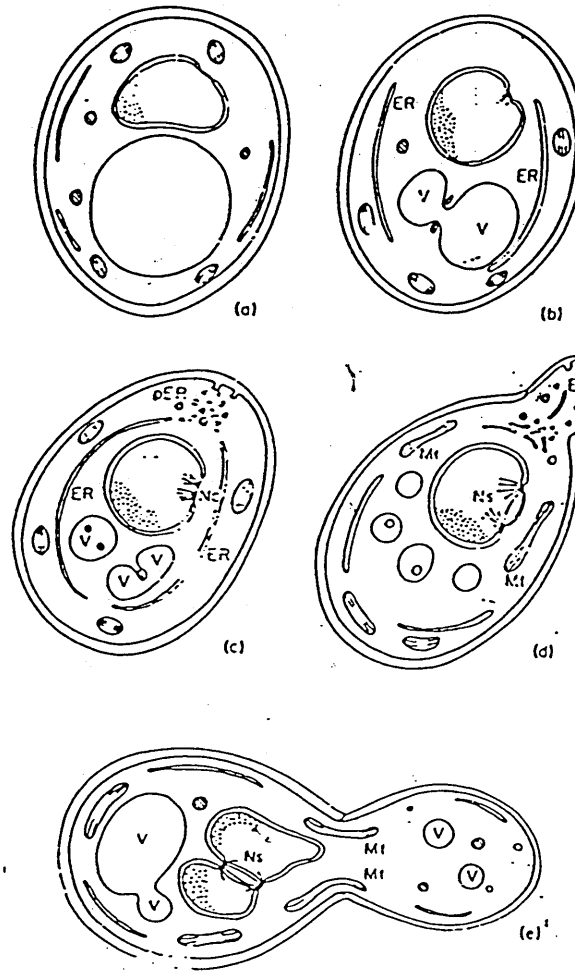
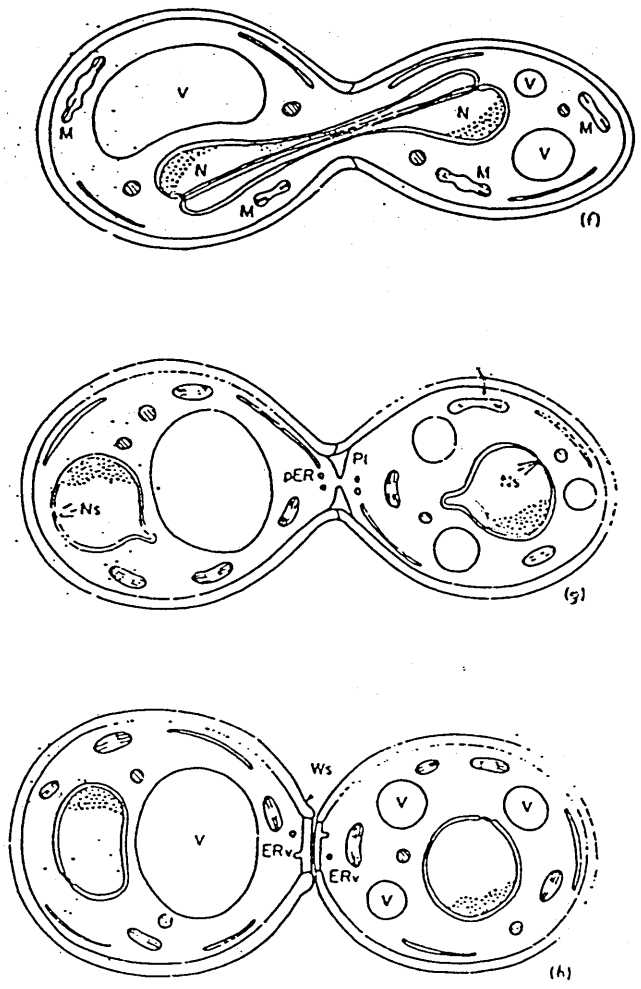


Diagram of the main cytological events in a budding yeast cell (Saccharomyces). (a) Resting cell. (b) Fusion of elements of endoplasmic reticulum, fission of the vacuole. (c) Continued fission of the vacuoles, proliferating endoplasmic reticulum, first endoplasmic reticulum-derived vesicles secreted through the plasmalemma, division of the centriolar plaque. (d) Formation of a budinitial, reduction of the endoplasmic reticulum, appearance of thread-like mitochondria, construction of the spindle apparatus. (e) Entry of vacuoles and thread-like mitochondria into the bud,



fusion of vacuoles in the mother cell, nucleus preparing to enter the bud. (f) Nuclear division, fragmenting mitochondria. (g) Plasmalemma closing the gap between mother cell and bud, proliferating endoplasmic reticulum, decay of the spindle. (h) Cross-wall formation, secretion of endoplasmic reticulum-derived vesicles. The mother cell contains one large, the daughter cell several small, vacuoles.

Figures on pages 32-34, A.H. Rose and J.S. Harrison, The Yeasts, 2 Academic Press (1971)

4.3 Yeast Fermentations

As outlined in Section 4.2.1 yeasts are capable of fermenting under a variety of conditions. This work is concerned with the highly aerobic fermentation of Saccharomyces Cerevisiae NCYC 990, however, some details will be given of the other fermentations.

Aerobic metabolism is directly affected by both available oxygen and sugars. For Saccharomyces Cerevisiae, in media with glucose concentrations above 5% (weight/weight), there is total inhibition of respiratory enzyme synthesis and hence the yeast will continue to ferment even in highly aerated media. This is referred to as the Crabtree effect. (35) In contrast, when growing in low glucose concentrations, approximately 0.1%, the yeast can change from anaerobic fermentation to respiration dependent upon aeration of the medium. This decrease in fermentative ability is known as the Pasteur effect (36).

In order to grow efficiently yeasts require oxygen, suitable temperature and pH, a readily utilisable carbon and nitrogen source, various minerals and other growth factors.

i) Carbon Source

All yeasts are able to utilise D-glucose, D-fructose and D-mannose. The ability to utilise a specific sugar depends upon enzymatic adaptability of the yeast. Saccharomyces Cerevisiae NCYC 990 can both ferment and assimilate a number of sugar sources including D-glucose. This latter sugar has been used as the carbon source throughout this work. Details of its concentration in the medium are given in Section 6.1.2 as are details of other components in the medium.

The carbon source is the main basis for energy production in the cell. Taking D-glucose as an example it would be utilised as follows. The sugar is tranported across the cell membrane by a

facilitated diffusion process. It is then phosphorylated by an hexokinase enzyme to glucose-6-phosphate. This in turn is converted to fructose - 1,6-diphosphate which in turn is converted by the Embden-Meyerhof pathway, (37) to pyruvic acid. This is decarboxylated to acetaldehyde and then to ethanol. The net result is that during the transformation of 1 mole of D-glucose, 2 moles of adenosine triphosphate (ATP) are produced. ATP is used as the main energy source for cell growth.

In addition to simple sugars, a number of other carbon sources can be utilised by yeasts. These include di-, tri- and oligosaccharides, as well as organic acids, alcohols, hydrocarbons, etc.

ii) Nitrogen Source

Yeasts have the ability to assimilate various compounds as a source of nitrogen. Saccharomyces Cerevisiae NCYC 990, in common with virtually all yeasts, can readily utilise ammonium sulphate as a nitrogen source, although the nitrate cannot be assimilated by most strains of Saccharomyces Cerevisiae.

Nitrogen is essential for yeast growth, acting as a building block for amino acids and proteins.

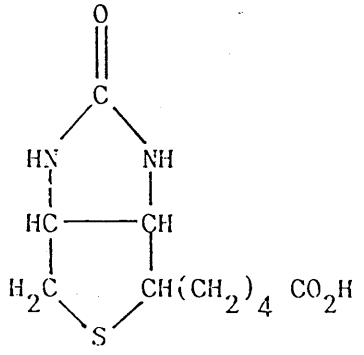
In addition to simple ammonium salts, amino acids can be assimilated. Surprisingly perhaps, the amino acids are not incorporated directly into proteins but are deaminated enzymatically.

Other compounds can act as nitrogen sources and a commonly used nitrogen source is urea.

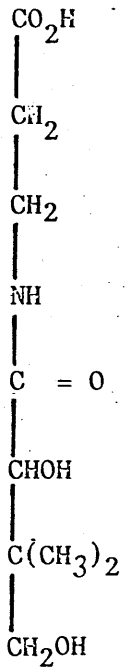
iii) Vitamins

The dependance of yeasts on an external source of vitamins varies widely from species to species. Vitamins are normally required as they form complexes with coenzymes and hence have catalytic functions within the cell. One vitamin has another role; meso-inositol is important as it is incorporated into phospholipids and is involved in membrane synthesis. However, Saccharomyces Cerevisiae NCYC 990 is not capable of assimulating inositol.

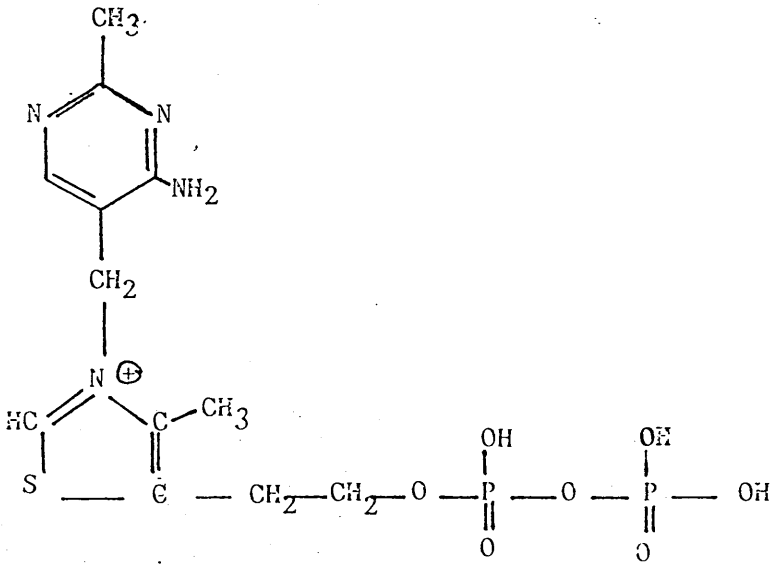
Biotin is the most commonly required vitamin for optimum growth. Its structure is shown below, as are those for Pantothenic acid, and Cocarboxylase which also enhance the growth of Saccharomyces Cerevisiae.



BIOTIN



PANTOTHENIC ACID



COCARBOXYLASE

iv) Minerals

Any of up to 50 elements can be found in yeasts in trace quantities. Their function is often obscure but some are considered vital for efficient growth. They are often utilised in enzyme and co-enzyme formation.

Inorganic mineral requirements in this work have been supplied by magnesium chloride, magnesium sulphate and phosphoric acid.

4.3.1 Fermentation Parameters and Principles of Aerobic Growth

Four main parameters; pH, dissolved oxygen level, Yeast yield and volume of vessel contents have been monitored in this work. These are discussed below, with details of other parameters being given.

i) Growth Rate

In most aerobic fermentations, the yeast cell mass shows exponential growth throughout the fermentation. For these fermatations the increase in cell mass can be expressed as the generation time or the specific growth rate constant for exponential growth. Generation time is that time taken for doubling cell mass. The specific growth rate constant (μ) is defined as follows:

$$\frac{dP}{dt} = \mu P \qquad \text{where } P = \text{mass of yeast}, \; t = \text{time}$$

upon integration at time t

$$\mu = 2.303 \log (Pt/Po)/t$$

hence the relation to generation time is:-

$$\text{Generation time (hr)} = \frac{2.303 \log_{10} 2}{\mu} = \frac{0.693}{\mu}$$

Obviously growth rate is also dependent upon availability of substrate. In this work D-glucose was fed at constant rate throughout 12 hour fermentations and hence during the fermentation, rate of growth fell because as cell numbers increase, the concentration of substrate required to maintain growth would also need to increase. In order to maintain constant growth rate, substrate would have to be added exponentially throughout the fermentation.

(ii) Oxygen Requirement

Oxygen availability is of vital importance for high yeast yields. Under strictly aerobic conditions the maxiumum theoretical yield coefficient (Ys) has been shown to be 0.54. This means a yield of 54 g of yeast per 100 g of fermentable sugar. (38)

In order to achieve high Ys values, μ must not exceed $2hr^{-1}$ and the respiratory quotient (RQ) should be approximately 1. RQ is given as $[CO_2] / [O_2]$. At higher growth rates carbon dioxide production is greatly accelerated as is ethanol production.

For most yeast fermentations, oxygen supply is in the form of sterile air. Not only is oxygen required but a suitable system to transfer oxygen from the air into the liquid phase is required. The capacity for transfer from gas to liquid phase is referred to as the oxygen transfer coeffient, K_{LA}. It is normally expressed in terms of millimoles of O_2/litre hour. Many factors affect this value, not least the interfacial surface area between gas bubbles and liquid. In order to achieve high oxygen transfer coefficients, maximum surface area of gas bubbles is needed. This is normally achieved by having mechanical agitation at a point near the base of a fermentation vessel where gas is introduced. This agitation results in physical disruption of large bubbles, with subsequent increase in the overall surface area. Various systems have been described for increasing the rate of oxygen transfer (39).

The level of dissolved oxygen during fermentations can be determined by oxygen electrodes (see Section 5.2.2). These show that during a yeast fermentation the dissolved oxygen concentration towards the end of a fermentation can fall to only 5% of initial figures. Hence oxygen supply can be a limiting factor on yeast yield. In this work oxygen supply was kept constant, again limiting yeast yields due to increasing cell numbers requiring increased oxygen concentration to maintain constant growth in the medium.

iii) pH and Temperature

Saccharomyces Cerevisiae can tolerate a fairly wide pH range and grows well at pH 3 - 6. Optimum pH for growth varies from species to species. Aerobic fermentations using Saccharomyces Cerevisiae result in the evolution of carbon dioxide which results in a fall in pH of the growth medium. This could obviously result in the pH falling below that optimum for growth. In most cases this is overcome by addition of alkali during the fermentation. Sodium or ammonium hydroxides are the bases most commonly used (40).

The optimum growth temperature for Saccharomyces Cerevisiae can fall anywhere in the range 20 - 40°C. However for most species the optimum growth tends to be at 28 - 30°C. At these temperatures it has been shown (41) that the yeast generation times are small, sometimes as low as 2 hours, with a resultant high yield. During the course of the fermentation heat is liberated as a result of sugar metabolism. Even for normal cell maintenance approximately 0.08 g sugar per g of yeast is used. Again if left unchecked, the evolution of heat could take the temperature of the fermentation medium above that for optimum growth. Cooling aids within the fermenter are used to maintain constant temperatures.

iv) Volume of Vessel Contents

One of the parameters of prime importance in this work was the change of volume of vessel contents during fermentation. This is dicussed in more detail in Section 4.5 and Section 5.2.1. However, mention of the need to monitor the volume of vessel contents is made briefly here.

As a direct result of both the introduction of oxygen into a fermentation medium and the subsequent evolution of carbon dioxide, there is considerable increase in the volume of vessel contents due to foaming. This causes problems in a number of ways including reduction of viable vessel capacity with a subsequent reduction in yield and also risk of loss of vessel contents and subsequent contamination of the yeast culture.

4.4 Foam in Fermentations

Although the direct consequence of foam in fermentations will be discussed later in this section, it is important first to consider what foam is and how it is caused. Some detail will then be given as to how foam can be controlled and how this in turn relates to foam in fermentations.

4.4.1 Foam and Foam Stabilisation

Foam can be considered to be a coarse dispersion of gas bubbles in a relatively small amount of liquid (42, 43). Stable foam cannot be produced from a pure liquid based on theoretical thermodynamic considerations. From the Gibbs function for a one component system (ie. a pure liquid).

$$dG = Vdp - SdT - \gamma dA$$

where γ is the surface tension of the liquid and A is the surface area per mole.
Integration gives

$$\Delta G = \gamma \Delta A$$

at constant temperature and pressure, where ΔG is the change in free energy of the system.

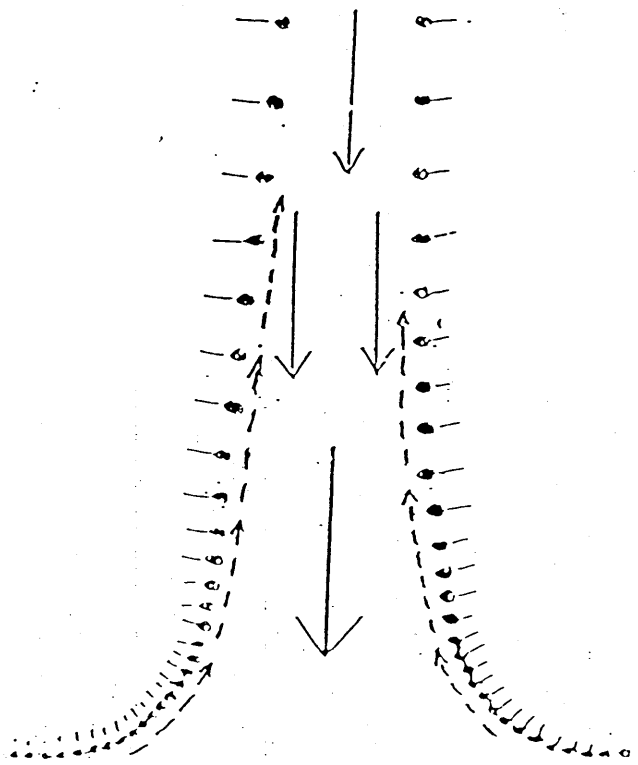
Hence a decrease of the Gibbs function would occur from a decrease in surface area which in turn could be caused by coalescence of the foam elements. Hence any foam produced in a pure liquid will only have transient existence.

In order for a foam to be produced with anything more than transient existence, the above equation requires additional terms to $\gamma\Delta A$ and also of opposite sign. This, in practice, is brought about by a solute that is positively adsorbed at the surface of the liquid and requires work to remove it from there back into the bulk of the liquid. If the gain in free energy resulting from this transfer is larger than $\gamma\Delta A$ then it is energetically favourable for foam to persist even though it may still be mechanically unstable.

In order to understand why foams persist and have mechanical stability other concepts need to be considered. The most relavant of these to this work are film elasticity and the formation of what are termed gelatinous surface layers.

Film elasticity can be considered to be the ability of a liquid film to resist localised thinning whilst general film thinning occurs. As the area of potential thinning starts to form the solute concentration will decrease with a subsequent increase in surface tension. As a result of the imbalance of surface tensions, the area surrounding the point of potential thinning, moves toward it to redress the balance. This movement of surface layer is accompanied by motion in underlying layers in the bulk liquid resulting in downward flow of liquid being counterbalanced to an extent by flow of liquid at the surface. The restoration of lost liquid was first described by Marangoni (44) and is known as the Marangoni effect.

A representation of the Marangoni effect is shown below:



The diagram represents the wall of a bubble. The surface films are concentrated with surface active agents (denoted ↓) and the drainage due to gravity is denoted by the large arrows (↓). As soon as the bubble is formed, drainage of liquid from the bubble wall begins to occur. This is a very large effect and the passage of liquid down the bubble wall pulls surface active molecules down the surface films. The concentration of surface active molecules becomes greater at the base of the surface film than at the top of the film. This in turn means that there is a lower surface tension at the base of the surface film than at the top. A locally high value of surface tension near low surface tension regions results in a surface tension gradient on the surface. This gradient forces a flow of surface active agents upwards in the surface films and these surface active agents in turn pull liquid molecules with them. This replenishment of surface active molecules along the surface film (denoted ↑) is called Maragoni motion and is the reason for the intial stabilisation of foam bubbles.

This effect was also recognised by Gibbs (45)

Although the first stabilising factor for a foam is the resilience provided by the Marangoni effect, other factors can be of vital importance in foam stability.

Foam life can be enhanced as a result of high bulk and surface viscosity. High viscosities can result if materials such as proteins are present. (This is of direct relevance in fermentation studies) High viscosity of the surface film results in a decrease in the rate of loss of liquid by purely hydrodynamic means. In some cases cessation of this loss occurs. In this form the surface layers are said to be gelatinous.

In addition to these factors Derjaguin and Titijevskaya (46) have suggested electrical repulsion between ionic double layers to be of importance in foam stability.

4.4.2 Control of Foam

Control of foam can be brought about by a number of mechanisms. Historically control of foam relied upon physical means to break bubble walls. A large number of mechanical devices have been designed whereby physical disruption of gas bubbles at the surface of a liquid occurs. However, although these devices succeed in disrupting the surface bubbles they usually do not thoroughly control foam as they do not prevent adsorption of solute at the liquid surface.

In addition to mechanical means, the use of water sprays have been considered to combat foaming when water based foams are encountered. They relied on the ability of water, sprayed on top of the foam to increase drainage rates, thus destabilising the foam and additionally the dilution effect resulted in reduction of surface viscosity. This in turn diminished the tendency to form gelatinous surface layers. However, water sprays are of only transient effectiveness as again ultimately they have no effect on migration of solute to the liquid surface.

The most effective control of foam is brought about by the use of chemical foam inhibitors, as is described below.

The use of chemical agents to both control foam and prevent its formation has been reviewed by Ross (42, 43). In order to control foam the agent must be able to destroy the surface elasticity and reduce the surface viscosity of the foaming system. To attain this the agent should in turn have low surface tension to enable it to spread on the foam lamellae and generally it should be insoluble in the foaming system.

Two main modes of action for these anti-foam agents have been considered by Ross. They first proposed that the droplet of antifoam enters the film between two bubbles and spreads as a relatively thick double layered film. The second idea was that the degree of spreading of the antifoam droplet on entering the film between the bubbles was more limited, resulting in the formation of a mixed monolayer, with the solute. It was postulated that as a result of poor coherence in this monolayer, the foam breaks.

Both mechanisms rely on the need for the antifoam to enter the film between two bubbles. This can be expressed in terms of an entering coefficient, E, and a spreading coefficient, S. They can be expressed as follows.

$$E = \gamma F + \gamma FA - \gamma A$$

$$S = \gamma F - \gamma FA - \gamma A$$

Where γF and γA are the surface tensions of the liquid and antifoam respectively and γFA is the interfacial surface tension between the two. In order to be effective the antifoam should have strongly positive entering and spreading coefficients. These equations thus illustrate the need for the antifoams to be of lower surface tension than the foaming medium. Some cases occur, where the interfacial surface tension is large, where E is positive and S is zero or negative. Antifoaming action in these cases is dependent upon poor film coherence in the mixed monolayer.

In order to ensure a large positive value for S, the value of γFA must be low. This usually implies the need for some hydrophilic character in the antifoam. This is somewhat in opposition to the requirement for insolubility. However, for most practical purposes antifoam compositions are a compromise between the requirements of insolubility and hydrophilicity.

4.4.3 Antifoam Types

Section 4.4.2 outlines that an antifoam should possess the following properties to be effective; low surface tension, low interfacial surface tension with the foaming medium, low solubility in the foaming medium and low cohesive forces in surface layers.

Many differing types of antifoaming agents are comercially available and have been used over the years. Often the surface tension of the foaming medium will dictate the choice of antifoaming agent. If the surface tension of the medium is, for example, 50 dyne cm^{-1} then the choice of antifoam is quite wide. In aqueous media simple glyceryl esters such as animal or vegetable oils will have sufficient insolubility and low surface tensions to be effective. However, if the surface tension of the system falls further, then these naturally occurring oils become ineffective as E becomes negative. In cases such as this synthetic antifoam agents are employed. Ross subdivided available types into eight main groups, these being:-

i)	Alcohols	eg. 2-ethylhexanol
ii)	Fatty acids and fatty acid esters.	eg. stearic acid, sorbitan trioleate
iii)	Amines	eg. diamyl amine
iv)	Amides	eg. stearamide
v)	Ethers	eg. p-tertiary amyl phenoxyethanol
vi)	Phosphate esters	eg. tributyl phosphate
vii)	Metallic soaps of fatty acids	eg. aluminium stearate
viii)	Silicones	eg. poly (dimethylsiloxanes)

The chemical types available are occasionally used alone or are compounded as mixtures for specific application. Historically in fermentation systems vegetable oils were commonly used. However they were succeeded by the silicones and latterly by high molecular weight polyethers. It is this latter type which are of interest in this work and they will be discussed in Section 4.5. However, mention will be made here of the silicone types.

Of the products previously used in fermentations, the poly (dimethylsiloxane) types were considered almost ideal antifoams. They have the general formula;

$$(CH_3)_3\ SiO\ [Si\ (CH_3)_2\ O]_n\ Si\ (CH_3)_2$$

Where n is an integer with values as high as 2,500.

They are colourless liquids with viscosities from 0.65 cP to greater than 10^6 cP. They are essentially insoluble in water and have surface tensions as low as 21 dyne cm^{-1}. They also possess extremely low surface viscosities. However, they do suffer from the disadvantage of having high interfacial surface tension (ca. 42 dyne cm^{-1}) with water. This results in a low spreading coefficient. These fluids cannot be used alone therfore and need some suitable hydrophilic material to improve their mobility in bubble lamellae. They are often used in the form of aqueous emulsions.

4.4.4 Foam in Fermentation Processes

In addition to the conditions giving rise to foam formation previously mentioned in Section 4.4.1, foaming during a fermentation process has the added complication that it changes during the course of the fermentation. The reasons for this are thought to be associated with cell activity, however little detailed information is available in the literature to confirm this.

Work on mycelial systems (47 - 50) has shown somewhat conflicting results. In one system a decrease in foam stability with time occurred, accompanied by an expected fall in surface viscosity and rise of surface tension. It is postulated that any foam stabilising components present in the initial medium were utilised by the mycelium. However, in a second mycelial system little change in surface tension occurred but a significant rise in surface viscosity was accompanied by an increase in foam stability, probably being in part due to formation of gelatinous surface layers.

This latter point may also be of importance to other fermentation parameters as there is some evidence (51) that viscous surface layers can present a barrier to carbon dioxide and oxygen absorption.

In addition to the above points Howe (52) has suggested a number of other factors which change foaming characteristics in a fermenting medium including changes in pH, temperature, growth rate, production of fatty materials, proteins etc.

4.4.5 Antifoams in Fermentation

Many types of antifoam are used in fermentation processes. Apart from the need to destabilise foam (see Section 4.4.2) they should also posses the following features.

i) Long lasting control of a constantly changing foam (see Section 4.4.4)
ii) Low toxicity to the micro-organism
iii) Limited effect on oxygen transfer rates
iv) Unable to be metabolised by the micro-organism

The need for these additional properties in an antifoam has further restricted the types that are used in fermentations. As a general rule few antifoams containing glycerides are used, as hydrolysis of the glyceride to fatty acid will cause a pH drop that could be undesirable to metabolism. Fatty alcohols and acids can also provide a barrier to carbon dioxide diffusion and these effect oxygen transfer rates (53).

Generally, silicones prove suitable for most fermentation systems, being of low toxicity. However, some evidence suggests they can change metabolic pathways in certain mycelia (54). They can also adversely affect oxygen transfer rates. These factors combined with the high aqueous interfacial surface tension values have led to the need for more suitable antifoams for use in fermentations.

To date the most likely candidate for an antifoam that will fulfil all the requirements for a fermention, is a group of compounds which would fall under section i) or v) of Ross' classification. These are polymers produced from ethylene oxide and propylene oxide. They are discussed in Section 4.5.

4.5 Ethylene Oxide - Propylene Oxide Copolymers as Antifoams

Ethylene oxide-propylene oxide copolymers are commercially available under the trade name Pluronic. They are of the general formula

$$H(O\text{-}CH_2\text{-}CH_2)_a(O\text{-}\underset{\underset{CH_3}{|}}{CH}\text{-}CH_2)_b(O\text{-}CH_2\text{-}CH_2)_aOH$$

It is possible to vary the values of a and b which in turn will modify the molecular weight and hydrophobicity of the polymer. This also affects its function as an antifoam.

The polymers are widely used as antifoam agents (55) in a number of applications. As a result of modifying the values of a and b it is also possible to modify the effect of the polymers on both surface tension and interfacial surface tension. Obviously the value of a and b can have a direct influence on the effectiveness of a particular polymer as an antifoam.

Full details on surface tension and interfacial surface tension values are shown in Appendix 2. However, brief details are given below to show how they may affect antifoam effectiveness. The variation in surface properties can be summerised as follows:

i) Increase in the value of b results in a decrease in aqueous surface tension.

ii) For a constant value of b, surface tension increases with increasing values of a.

iii) Increase in the value of b results in a decrease in interfacial surface tension.

iv) For a contant value of b, interfacial surface tension increases with increasing values of a. This effect is less pronounced than that outlined in ii) for surface tension.

Generally these polymers do not exhibit the very low aqueous surface tensions of the poly(dimethylsiloxances) but still show values as low as 32.5 dyne cm^{-1}. However, one of the main contributary factors to the use of the ethylene oxide-propylene oxide copolymers as antifoams is their low interfacial surface tensions in aqueous systems. In an aqueous nujol system some of the polymers exhibit interfacial surface tensions as low as 1.8 dyne cm^{-1}.

The table below indicates how the values for interfacial surface tension can affect antifoaming properties for a series of polymers containing a set number of propylene oxide units in an aqueous system with a surface tension of 72 dyne cm^{-1}, and a polymer concentration of 0.001% w/w.

Molecular Weight Mn	Value of b	Value of a	Surface Tension/ dyne cm^{-1}	Interfacial Surface tension/ dyne cm^{-1}	E/dyne cm^{-1}	S/dyne cm^{-1}
2022	30	3	47.2	19.5	44.3	5.3
2550	30	9	48.5	20.5	44.0	3.0
2902	30	13	49.0	21.5	44.5	1.5
8358	30	75	53.6	26.0	44.4	-7.6

Thus, although it can be seen that all of these polymers have strongly positive entering coefficients, their ability to spread in the bubble lamellae decreases with increasing value of a. With a value for a of 75, S becomes negative and the polymer will not spread within the lamellae. This latter polymer would not be an effective antifoam for this system.

In addition to these points, the aqueous solubility of the polymers has to be considered when examining antifoam suitability. Poly(oxypropylene) polymers with molecular weights of 900 and more are essentially water insoluble. However incorporation of oxyethylene units into the polymer

chain increases aqueous solubility. Ethylene oxide-propylene oxide copolymers with more than 30% w/w oxyethylene units are soluble in water at 20°C at concentrations of 10%. The polymers also show decreased solubility with increase in temperature.

Regarding toxicity of these polymers to various micro-organisms little published data are available (56-58). However the data that is available suggest that the polymers are relatively inert toward breakdown by micro-organisms. This however appears to be partially dependent upon molecular weight of the polymers, with lower molecular polymers being more prone to degradation. Very limited information is available that suggests the polymers are non-toxic to micro-organisms. However their use in a number of fermentations (59) suggests they are and it is part of this work to examine their effect on yeast yield in fermentation.

Concerning affect on oxygen transfer rates, conflicting information is available (60-63). Some data suggests that most surface active agents have a deleterious affect on oxygen transfer rates by causing bubble coalescence and decreased aqueous oxygen solubility, whereas other data suggests little effect on oxygen transfer. Again investigation of the effect of ethylene oxide-propylene oxide copolymers on dissolved oxygen concentrations was studied in this work.

It can be seen that, on the basis of the available data, these polymers potentially fulfil a number of requirements for an antifoam for use in yeast fermentation.

4.6 Detection of Ethylene Oxide-Propylene Oxide Copolymers

Ethylene oxide-propylene oxide copolymers are essentially based on a hydrophobic poly(oxypropylene). As more oxyethylene units are added the resultant polymers become progressively more hydrophilic. The change in hydrophobic/hydrophilic balance affects how the polymers migrate to an air/water interface. On the basis of this it is also likely that this balance will affect how the polymers migrate to other interfaces such as that of yeast cells in an aqueous suspension.

It is thought that migration of the polymers onto the yeast cell could change the rate of yeast growth by a variety of mechanisms eg. acting as a physical barrier to oxygen uptake, being taken into the cell and affecting cell metabolism etc. As well as the possiblility of changing the rate of yeast growth, removal of polymer out of the aqueous phase onto the yeast cell will effectively reduce the available polymer concentration in the aqueous phase and in turn may change its efficiency as an antifoam.

In order to try to correlate these effects it was essential to develop a method to detect ethylene oxide-propylene oxide copolymers in aqueous media. This would enable the concentration of polymer remaining in the aqueous phase at the end of the fermentation to be determined, thus enabling the quantity of polymer absorbed on the yeast to be calculated.

Two approaches were initially considered for polymer detection; physical and chemical. The physical method relied on correlation of surface tension reduction of the aqueous media with polymer concentration. A number of chemical methods have been evaluated including pyrolysis, iodiometric analysis, complexiметric methods etc. However, the most reproducible quantitative method was based on a complexiметric technique developed by Schoenfeldt (64). It produced linear relationships between polymer concentration and complexing reagent. These methods are described in Section 6.4 and discussed in Section 5.3.

4.7 Aim of the Present Work

The aim of the present work was to purify and characterise the commercially available ethylene oxide-propylene oxide copolymers with respect to molecular weight and poly(oxyethylene) content. It was then intended to relate the variation in molecular weight and poly(oxyethylene) content of the polymers with a number of fermentation parameters in an aerobic fermentation of the yeast, Saccharomyces Cerevisiae NCYC 990. The parameters examined were yeast yield, pH, dissolved oxygen concentration and variation of the volume of vessel contents.

By relating these parameters to molecular weight and poly(oxyethylene) content of the polymers it was hoped to define a specific polymer as being the most effective for the particular fermentation ie. a polymer that could effectively control foam formation during the course of the fermentation and would not adversely affect the rate of growth of the micro-organism.

Chapter 5 Results and Discussion

5.1 Cultures of Saccharomyces Cerevisiae NCYC 990

5.1.1 Pre-Cultures

Saccharomyces Cerevisiae NCYC 990 was obtained from the National Collection of Yeast Cultures. The yeast was supplied freeze dried in sterile ampoules. The technique which was employed for forming pre-cultures is described in Section 6.1.1. The freeze dried yeast was aseptically transferred into sterile Difco YM broth. This broth contains glucose as a carbohydrate source, peptone as a nitrogen source and yeast extract as a source of B vitamins and growth promoters.

The culture was incubated at 28°C, pH 6.2 for 7 days. This yeast was then successively sub-cultured in Difco YM broth for a further 7 days in order to obtain sufficient yeast for fermentation studies. Sub-culturing of the yeast was carried out weekly throughout the course of the work in order to provide a continual source for fermentation studies.

After subculturing, the yeast was separated from the broth by centrifugation, washing the deposit with sterile distilled water and further centrifugation. This yielded a yeast paste with a solids content of approximately 20% which was used in the fermentation work. The yeast produced by sub-culturing was pale cream in colour and of consistent texture showing no signs of flocculation.

5.1.2 Medium for Fermentation Studies

After separation from the pre-culture, the yeast was introduced into a medium with the following composition.

D-Biotin	:	0.1 mg
$MgCl_2.6H_2O$	:	0.15 g
$MgSO_4.7H_2O$	:	0.05 g
Pantothenic Acid (Ca^{2+} salt)	:	0.01 mg
Aneurine Hydrochloride	:	1.0 mg
Cocarboxylase	:	1.0 mg
NH_4OH (0.88)	:	3.0 cm^3
H_3PO_4 (85%)	:	0.25 cm^3
Distilled water	:	to 1000 cm^3

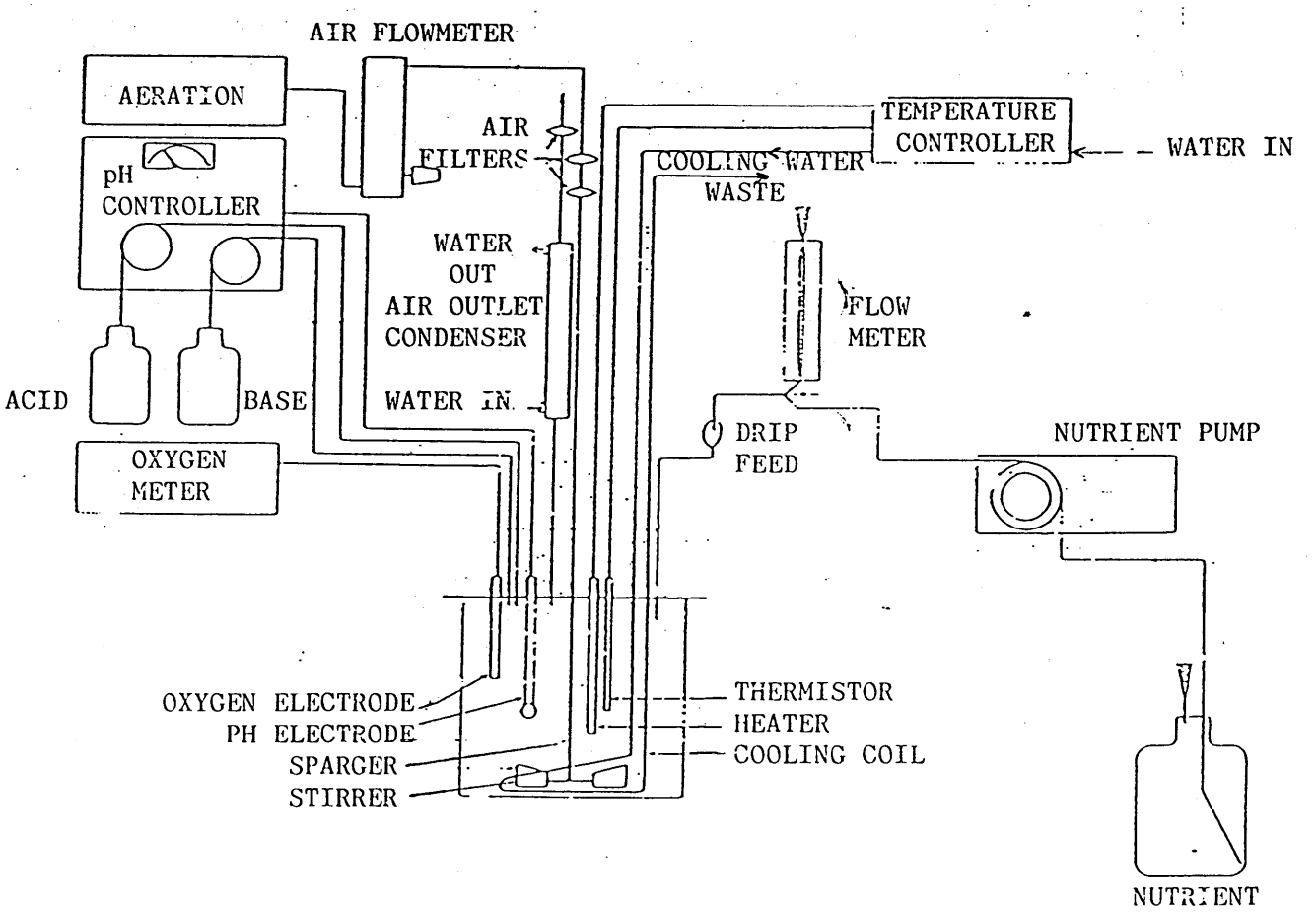
pH adjusted to 4.3 with H_2SO_4 (25% w/w)

For the fermentation studies 0.7 g (dry) yeast was introduced into 500 cm^3 medium in the fermenter, to which 3 cm^3 D-glucose (50 g dm^{-3}) had been added. To this medium D-glucose was added throughout the fermentation at a rate of 6 cm^3 hr^{-1} (50 g dm^{-3} D-glucose). The fermentation was carried out for 12 hours and thus a total of 3.75 g D-glucose was available for metabolism by the yeast.

Prior to use both the medium and D-glucose solutions were sterilised at 121°C and 15 psi for 30 minutes and 15 minutes respectively.

5.2 Fermentation Apparatus and Parameters

A more detailed description of the fermentation apparatus is given in Section 6.2. However a schematic representation is show below.



Figure; Gallenkamp, Modular Fermenter Handbook, Publication 713, 2M/1/78 (1978)

A brief description of the apparatus is as follows: The fermentation vessel had a capacity of 1000 cm^3. The vessel complete with all of the probes shown above was sterilised (121°C, 15 psi, 60 minutes) prior to the fermentation commencing.

After preparation of the apparatus as shown above, fermentations were carried out in the following manner. The fermentation medium (500 cm^3) was poured into the vessel. To this Saccharomyces Cervisiae NCYC 990 (0.7 g dry) was added as a 25% w/w dispersion in sterile distilled water. This was immediately followed by addition of sterile ethylene oxide-propylene oxide copolymer. The polymer was previously sterilised under UV light (254 nm) for 15 minutes. The pH of the aqueous phase was adjusted to 4.5 - 4.6. The following conditions were then used throughout the fermentations.

Stirrer speed:	600 rpm
Aeration rate:	500 $cm^3\ min^{-1}$
Nutrient feed rate:	6 $cm^3\ hr^{-1}$ (50 g dm^{-3} D-glucose)
Fermentation time:	12 hours
Temperature:	30.2 $\pm$ 0.2°C

During all of the fermentations, the pH fell. However by automatic addition of 2% w/w sodium hydroxide it was never allowed to fall below 3.65. Below this pH growth rate of the yeast was markedly reduced.

Throughout the fermentations, pH, dissolved oxygen content, temperature and the volume of the vessel contents were recorded every 30 minutes for the first two hours, and then hourly for the next 10 hours.

At the end of the fermentation, the vessel was emptied and the culture centrifuged. The aqueous phase was retained in order to ascertain the concentration of ethylene oxide-propylene oxide copolymer remaining in it. The yeast phase was washed and centrifuged again. The dry weight, non-volatile content and appearance of the yeast was noted.

5.2.1 Fermenter Contents Volume

The effect of the polymers on the fermenter contents volume was examined with reference to a fermentation with no polymer present. The polymers were examined at five different addition levels from 5 mg to 100 mg. In each case the fermentation was carried out in triplicate. The mean results showing variation of the vessel contents volume with time are shown graphically in appendices 3 - 12 and in tabular form, appendices 13 - 21. The results for each polymer examined are discussed below.

5.2.1.1 No Polymer Present

In the absence of any polymer, increase in the vessel contents volume was directly associated with two factors.

i) As with all of the fermentations examined, there was a controlled increase in vessel contents resulting from the continuous addition of D-glucose solution during the fermentation. The addition rate was 6 cm^3 hr^{-1} which would represent an increase in volume of 72 cm^3 after a 12 hour fermentation. Thus in the absence of other factors the final vessel contents volume would have been 622 cm^3 (Although the actual liquid volume was only 572 cm^3, liquid displacement due to the presence of the stirrer, thermometer, probes etc. represented a further 50 cm^3).

ii) As a result of the high agitation and aeration rates, large numbers of air bubbles were generated in the aqueous medium. In the absence of any stabilising factors these bubbles would have destabilised at the air liquid interface and not led to any appreciable volume increase. However, with a fermentation containing yeast a number of potential stabilising factors were present. These included the yeast cells themselves as well as any water soluble metabolites. These factors markedly increase the stability of bubbles at the interface by either reducing drainage or by increasing surface viscosity. As can be seen in appendices 3 and 13 these factors resulted in a marked increase in the vessel contents volume with time. In these fermentations the vessel contents volume increased such that the vessel contained greater

than 1000 cm^3 within 4 hours of the fermentation starting. In order to maintain these fermentations it was necessary to withdraw stabilised foam from the surface of the fermentor periodically. At the end of the 12 hour fermentation this amounted to no more than 50 cm^3 of the culture. However, this rapid and continuous increase in vessel contents volume was clearly undesirable. It would have resulted in poor utilisation of vessel capacity as well as increasing the risk of contamination due to blockage of sterile filters by foam, sub-culturing of the yeast on dried foam on the top of the fermenter etc.

It was apparant that in order to carry out a fermentation for 12 hours, the presence of bubble destabilising factors was needed. The results of the addition of various ethylene oxide-propylene oxide copolymers as destabilising agents are shown below.

5.2.1.2 Pluronic L31P

This polymer was previously characterised by ^{13}C NMR and GPC as having a polyoxyethylene content of 10.9% (w/w) and a weight average molecular weight of 974.

The results of the fermentations carried out in the presence of Pluronic L31P are shown in appendices 4 and 13.

It was seen that at all of the concentrations studied, the vessel contents volume never reached 1000 cm^3 at the end of the 12 hour fermentation period. The polymer appeared to be capable of destabilising the foam produced. However, this effect did appear to be concentration dependent as shown below.

Polymer Concentration (gdm^{-3})	Increase in Vessel Contents Volume After 12 Hour Fermentation (%)
0.010	61
0.030	52
0.100	46
0.150	43
0.200	36

At the highest concentration studied (0.2 gdm^{-3}) the rate of volume increase was almost linear throughout the fermentation period. However as the concentration of polymer was decreased, the rate of volume increase became non-linear. At the two lowest concentrations studied the rate of volume increase over the first four hours of the fermentation was almost equivalent to that shown after twelve hours for the three higher concentrations.

In view of the fact that Pluronic L31P has a relatively high surface tension (see appendix 2) it was somewhat surprising that the percentage increase in the vessel contents volume was as low as shown. This unusual finding was further compounded by the fact that Pluronic L31P

had a 1% (w/w) cloud point of 32.5°C in the fermentation medium. (The cloud point of an ethylene oxide-proplylene oxide copolymer is that temperature at which an aqueous solution of the polymer becomes cloudy upon heating. Below this temperature the polymer is essentially soluble. The phenomenom is due to dehydration (65). Normally the ether linkages within the polymer are hydrated. However, upon heating the energy of the hydrogen bonding (approx 7 kcalmole^{-1}) is insufficient to retain the water molecules and thus the polymer becomes insoluble).

Therefore, as the fermentations were carried out at 30.2 $\pm$ 0.2°C, Pluronic L31P should have been soluble in the fermentation medium at the start of the fermentation. As dicussed in Section 4.4.2, an antifoam should preferably be insoluble in the foaming medium to be effective. It was not known whether Pluronic L31P became progressively more insoluble in the medium as the fermentation progressed.

It was postulated that the effectiveness of Pluronic L31P in the system could be attributed to the reduction in surface tension of the medium and the highly positive entering coefficient (approximately 43 dyne cm^{-1}). Although Pluronic L31P has a highly negative spreading coefficient (approximately -10 dyne cm^{-1}) it would be able to enter the bubble film but would not spread on it. However it appeared to sufficiently increase surface drainage as to cause bubble destabilisation. Its efficiency as an antifoam was somewhat limited, especially at the lower concentrations studied. This could be attributed to both its solubility and low spreading coefficient.

5.2.1.3 Pluronic L61P

This polymer has a weight average molecular weight of 2271 and a polyoxyethylene content of 11.0%

The results showing variation of vessel contents volume with time for fermentations carried out in the presence of Pluronic L61P are shown in appendices 5 and 14.

Again for all concentrations examined the volume of vessel contents never exceeded 1000 cm^3 after a 12 hour fermentation. The percentage increase in vessel contents volume after 12 hours were as follows.

Polymer Concentration (gdm^3)	Increase in Vessel Contents Volume After 12 Hour Fermentation (%)
0.010	41
0.030	30
0.100	29
0.150	24
0.200	21

The volume of D-glucose solution added to the fermentations represented a 13% increase in volume. Thus it can be seen that even at the lowest concentration evaluated, 0.010 gdm^{-3}, control of foaming by Pluronic L61P was very marked in comparison to a polymer free system showing an increase in volume due to foam of only 28%.

Pluronic L61P is based on a higher molecular weight polypropylene glycol than Pluronic L31P and so would be expected to be more hydrophobic despite a similar percentage polyoxyethylene content. This is illustrated by the fact that Pluronic L61P had a cloud point of 22.1°C in the initial fermentation medium and so would have been insoluble at the fermentation temperature. The polymer also has lower surface tension and interfacial surface tension values than Pluronic L31P. This results in a highly positive entering coefficient (approximately 44 dyne cm^{-1}) and also a positive spreading coefficient (approximately 6 dyne cm^{-1}). (See Apppendix 2)

These facts suggest that this polymer would be an effective antifoam ie. it was insoluble in the system and has the ability to enter into and spread on the bubble lamellae. The results obtained in the fermentations confirm this.

5.2.1.4 Pluronic L62P

This polymer with a weight average molecular weight of 2363 and a polyoxyethylene content of 23.5% was derived from the same molecular weight polypropylene glycol as Pluronic L61P, as were Pluronic L63P and L64P.

Pluronic L62P was also capable of maintaining vessel contents volume below 1000 cm^3 for all concentrations studied although it was less effective than Pluronic L61P. The results showing the vessel contents volume variation are shown in appendices 6 and 15. The results below show that at the lower concentrations studied this polymer was not a particularly effective antifoam.

Polymer Concentration (gdm^{-3})	Increase in Vessel Contents Volume After 12 hour Fermentation (%)
0.010	76
0.030	67
0.100	51
0.150	44
0.200	41

The results in appendix 6 show that over all of the concentrations studied control of foam was good for the first 8 hours of the fermentation. After this period only a 33% increase in volume occurred even at the lowest concentration studied. However, over the next 4 hours a rapid deterioration of foam control occurred.

Pluronic L62P had a cloud point of 24.0°C in the fermentation medium. This combined with a slightly less positive spreading coefficient (approximately 2 dyne cm^{-1}) would suggest that this polymer should be less effective than Pluronic L61P at inhibition of foaming. The results confirm this. It is considered likely that the higher polyoxyethylene content is attributable for the reduction in effectiveness in this case.

5.2.1.5 Pluronic L63P

This polymer has a polyoxyethylene content of 26.1%. This was somewhat lower than expected as the original polymer from which it was derived, Pluronic L63, should have polyoxyethylene content of 30 - 40%. The weight average molecular weight of Pluronic L63P is 2273.

Over the range of concentrations studied Pluronic L63P prevented the vessel content values exceeding 1000 cm^3 after a 12 hour fermentation as can be seen below.

Polymer Concentration (gdm^{-3})	Increase in Vessel contents Volume After 12 hour Fermentation
0.010	58
0.030	53
0.100	51
0.150	48
0.200	45

These results were similar to those obtained with Pluronic L62P, although at the lower concentrations studied Pluronic L63P appeared more effective at controlling foaming. It is not surprising that the results were similar in view of the similarity in molecular weight and polyoxyethylene content of the two polymers.

Pluronic L63P had a lower interfacial surface tension, than Pluronic L62P, giving a spreading coefficient of approximately 4 dyne cm^{-1}. This may explain the improved efficiency of this polymer at the lower concentrations studied.

5.2.1.6 Pluronic L64P

This polymer has a weight average molecular weight of 2477 and a polyoxyethylene content of 38.9%.

At all the concentrations studied the volume of vessel contents exceeded 1000 cm^3. It would have appeared that this polymer may actually have enhanced foam formation in the medium.

This is not surprising as it was soluble in the medium at the start of the fermentation (cloud point 37.6°C). Although it caused a reduction in surface tension of 16.5 dyne cm^{-1}, its relatively high interfacial surface tension results in a negative spreading coefficient.

Increase in the vessel contents volume with time was linear which pointed to bubble stabilisation due to the presence of the polymer, in addition to increase of yeast cell numbers with time.

It was apparent that Pluronic L64P was not an effective antifoam for this fermentation mainly due to its increased hydrophilic character in comparison to Pluronic L61P, L62P and L63P.

5.2.1.7 Pluronic L81P

This polymer has a weight average molecular weight of 2814 and a polyoxyethylene content of 14.6%. It was derived from a higher molecular weight polypropylene glycol than those polymers previously discussed.

For all of the concentrations studied the vessel contents volume never exceeded 1000 cm^3 during the 12 hour fermentation. The increases in the vessel contents volume were as follows.

Polymer Concentration (gdm^{-3})	Increase in Vessel Contents Volume After 12 hour Fermentations (%)
0.010	44
0.030	31
0.100	30
0.150	25
0.200	23

This polymer gave good control of foaming throughout the fermentation. Foam volume gradually increased during the fermentation in line with increase in yeast cell mass. Pluronic L81P was insoluble in the fermentation medium (cloud point 19.2°C). At the lowest concentration evaluated it caused a 23.4 dyne cm^{-1} reduction in surface tension. However the polymer had a far lower interfacial surface tension than those previously discussed, resulting in an entering coefficient of 49 dyne cm^{-1} and a spreading coefficient of approximately 9 dyne cm^{-1}. The effectiveness of this polymer appeared similar to that of Pluronic L61P although it was marginally less effective than its lower molecular weight analogue.

Data obtained from surface tension measurements suggests that the polymer should be an effective antifoam. This was found to be the case.

5.2.1.8 Pluronic L92P

This polymer has a weight average molecular weight of 3427 and a polyoxyethylene content of 21.2%.

In all of the fermentations studied, the use of this polymer was not sufficient to maintain the vessel contents volume below 1000 cm^3 for a 12 hour fermentation.

The results in appendix 10 show that the best results, in terms of foam control, were obtained when the lowest concentration of polymer was present, 0.010 gdm^{-3}. Using this concentration vessel contents did not exceed 1000 cm^3 until the fermentation had been in progress for almost seven hours. When the concentration of polymer was increased vessel contents volume increased more rapidly to the extent that at the highest polymer concentration, 0.200 gdm^{-3}, the contents of the vessel exceeded a volume of 1000 cm^3 after only one hour.

Data from surface tension measurements tended to suggest that the polymer should have been effective at inhibiting foaming. Surface and interfacial surface tensions were close to the values obtained for Pluronic L81P. However Pluronic L92P had a higher cloud point of 28.1°C. It was perhaps this fact which may be attributable to the higher polyoxyethylene content of the polymer, which may have given rise to its lack of effectiveness in controlling foaming. It is possible that as inorganic salts were removed from the fermentation medium by the yeast for use in protein synthesis and cell growth, the resulting fall in ionic strength of the medium resulted in the polymer having a raised cloud point (ie. above the fermentation temperature) at the latter stages of the fermentation. It was not possible to verify this as cloud points were very difficult to determine in the final medium due to turbidity of the medium even after filtration.

Thus for this polymer a disparity existed between anticipated results from surface tension data and those results obtained in the fermentations.

5.2.1.9 Pluronic L101P

This polymer has a polyoxyethylene content of 12.9% and a weight average molecular weight of 3893.

The polymer was very effective at controlling foaming as can be seen below.

Polymer Concentration (gdm^{-3})	Increase in Vessel Contents Volume After 12 hour Fermentation (%)
0.010	40
0.030	39
0.100	33
0.150	30
0.200	28

As with most of the other polymers studied, the control of foaming was improved with increasing polymer concentration. Surface tension data gave rise to highly positive entering and spreading coefficients. This combined with a cloud point of only 16.3°C would suggest that this polymer should have been highly effective at foam inhibition.

Data obtained from the fermentations supports this. It is also interesting to note that even for the lowest concentrations studied, any foam produced during the fermentation had only transient stability. At the end of the fermentation, when aeration and agitation ceased, the foam collapsed completely within five seconds whereas with more hydrophilic polymers such as Pluronic L64P the foam was present for several minutes.

5.2.1.10 Pluronic L121P

This polymer, with a weight average molecular weight of 4027 and polyoxyethylene content of 12.6%, was the most hydrophobic of those studied.

Over all concentrations studied control of foaming was excellent as shown below.

Polymer Concentration (gdm^{-3})	Increase in Vessel Contents Volume After 12 hour Fermentation (%)
0.010	33
0.030	31
0.100	31
0.150	29
0.200	26

Little variation in effect was apparent over the concentration range studied with an actual increase in volume of only 20% being shown at 0.010 gdm^{-3}. It was not possible to extend the range of concentrations studied below 0.010 gdm^{-3} due to difficulties in adding less than 5 mg polymer to the vessel.

The results obtained agree with surface tension data suggesting an entering coefficient of 46.3 dyne cm^{-1} and a high spreading coefficient of 27 dyne cm^{-1}.

As with Pluronic L101P any foam produced only had transient existence.

5.2.1.11 Summary

The polymers examined can be divided into two main groups

a) Those derived from the same molecular weight polypropylene glycol base ie. Pluronics L61P, L62P, L63P and L64P.
b) Those with similar polyoxyethylene contents ie. Pluronics L31P, L61P, L81P, L101P and L121P.

Pluronic L92P cannot be readily classified into either of these groups.

Regarding the polymers in group a), it would appear that as the polyoxyethylene content increases and the polymers become more hydrophilic, their effectiveness at controlling foaming is reduced. This is in line with the reduction in spreading coefficient as polyoxyethylene content increased. Thus for this particular range of polymers, the lowest molecular weight most hydrophobic member, Pluronic L61P, was the most effective polymer with respect to foam control.

Regarding polymers in group b) these all had similar polyoxyethylene contents (10.9 - 14.6%). At the lowest concentrations studied, 0.010 gdm^{-3}, it was found that an increase in molecular weight of the polymer resulted in more effective control of foaming. However at higher concentrations little difference in efficiency was observed. All of the polymers with polyoxyethylene contents between 10.9 and 14.6% were very effective antifoams.

Overall Pluronic L61P and L121P gave most effective control of foaming.

5.2.2 Dissolved Oxygen

Before the fermentations were started, the culture medium was saturated with oxygen. This was done by agitating the medium at 600 rpm in addition to passing air through it at 500 cm^3 min^{-1}. The air introduced into the fermenter was sterilised by passing it through two microbiological filters in series. Dorman (66) showed that for two such filters in series $10^{10} m^3$ air would have to pass before one organism penetrated them.

When the culture medium, at 30°C, was saturated with oxygen this represented a concentration of 7.5 mg dm^{-3} of oxygen. The concentration of oxygen in the culture medium was recorded by means of an oxygen electrode. The detailed use of this electrode is described in section 6.2.3. The electrode relies on the Mackereth principle (67) which is briefly described as follows. The Mackereth cell consists of a perforated silver cathode and a lead anode immersed in an alkaline electrolyte. The cathode is surrounded by a membrane of polyethylene or polytetrafluoroethylene which is selectively permeable to oxygen. No applied potential is required and diffusion of oxygen into the cell results in a galvanic current proportional to the concentration of oxygen in the aqueous medium. The electrode reactions are:

At the anode: $2Pb^{o} + 6OH^{-} \longrightarrow 2PbOOH^{-} + 2H_2O + 4e$

At the cathode: $O_2 + 2H_2O + 4e \longrightarrow 4OH^{-}$

giving an overall cell reaction:

$$2Pb^{o} + 2OH^{-} + O_2 \longrightarrow 2PbOOH^{-}$$

Thus the current measured is determined by the rate of diffusion of oxygen through the membrane and as such is a function of the concentration of oxygen in the aqueous phase.

The output from the electrode was relayed to a dissolved oxygen meter calibrated in percentage terms. In general the oxygen solubility in the medium was not known as it depended upon the ionic strength of the medium which varied during the fermentation. Solutions of varying ionic strength had differing oxygen concentrations after air saturation (68). The electrode was calibrated at 100% after saturation with air. The electrode was also calibrated at 0% after oxygen free nitrogen was sparged through the medium at 30°C under agitation at 600 rpm until the medium was saturated with only nitrogen.

The method of introducing oxygen into the medium was of prime importance in this work as oxygen availability was vital to the fermenting yeast. Aeration was achieved by both vortex action and by sparging and is described in section 6.2.3. A vortex system was employed to draw air down from the headspace above the medium and disperse it in the medium by action of the centrifugal impeller blade. In addition air was sparged into the medium by means of a capillary tube positioned so that air bubbles were introduced directly adjacent to the impeller which had blades on the underside to ensure good air distribution.

For all the fermentations studied the medium was saturated with air prior to fermentation to give a reading of 100% at 30°C. Yeast was then introduced into the fermenter which resulted in a fall in dissolved oxygen levels within 30 seconds of addition. The falls in dissolved oxygen levels at this stage were typically only 1%. For all of the fermentations studied dissolved oxygen levels continued to fall as the fermentation proceded. Initially it was hoped that it would be possible to quantify the relationship between rate of fall of dissolved oxygen and the antifoam concentration. However a number of factors resulted in this not being possible. As was mentioned above the change in ionic strength during the fermentation would result in changes in dissolved oxygen concentration as would changes in pH (69). However the main

factor limiting the scope of the quantitative information obtained from the oxygen electrode was that the membrane itself was prone to fouling by the yeast. During the fermentation, as the concentration of yeast cells increased, deposition of the organism onto the solid surfaces in the culture vessel occurred including the oxygen electrode. This layer of cells effectively acted as a partial barrier to oxygen diffusion and could have caused erroneous quantitative information. Thus any information obtained using the oxygen electrode can only be discussed in general terms as follows. The results for dissolved oxygen levels are shown in appendices 22 - 41.

5.2.2.1 No Polymer Present

When no polymer was present in the fermentation medium the dissolved oxygen concentration fell gradually throughout the fermentation eventually falling to 58% of the initial saturation. The rate of decline in dissolved oxygen level was most marked during the first two hours of the fermentation. This can be associated with rapid rates of increase of cell numbers as availability of glucose is not a limiting factor. However as the fermentation progresses glucose availability does become the limiting factor. This is associated with the fact that glucose is added to the fermenter at a fixed rate whereas cell numbers increase exponentially. Hence towards the end of the fermentation glucose availability is limited and it would be anticipated that the rate of fall in dissolved oxygen levels would become reduced. This agrees with results shown in appendices 22 and 41.

If glucose availability had not been limited previous work (70) has shown that dissolved oxygen levels can fall to less than 5% of initial saturation values and in these fermentations oxygen would be the factor limiting yeast yield. In this fermentation when no polymer was present the values for dissolved oxygen concentration may also have been affected by the presence of stabilised foam above the medium which could be a barrier to both carbon dioxide and oxygen release from the medium. Chain and Gualandi (71) have reported this as a factor in increasing oxygen absorption rates in fermentations.

5.2.2.2 Pluronic L31P

The effect of this polymer, as with all the others examined, on dissolved oxygen concentration was evaluated at five addition levels, 5, 15, 50, 75 and 100 mg in 500 cm^3 medium. The results are shown in appendices 23 and 32.

At the two lowest addition levels, Pluronic L31P appears to have little effect on dissolved oxygen concentration in comparison to a fermentation with no polymers present. When Pluronic L31P was added at 5 and 15 mg to the initial medium, dissolved oxygen levels fell with time eventually falling to 44% and 47.5% respectively at the end of the twelve hour fermentation. However when the concentration of polymer was increased the effect on dissolved oxygen concentration was more marked. At the three highest concentrations studied the dissolved oxygen concentration had only fallen to 82%, 86.5% and 90% at the end of the 12 hour fermentation.

Two interpretations could be placed on these results. The first is that at the higher polymer concentrations studied, the polymer is being absorbed on to the surface of the yeast cells and as such is acting as a physical barrier, preventing oxygen being taken into the cell. In this case it would be anticipated that the decrease in dissolved oxygen concentration would fall with increasing polymer weight. It is also likely that this effect would result in a decrease in yeast yield at the end of the fermentations as less oxygen would be available to increase cell mass. This is discussed in section 5.2.5.2.

A second explanation relates to the efficiency of the polymer as an antifoam. Results in section 5.2.1.2 showed that as the concentration of polymer increased so did its efficiency as an antifoam. As antifoam efficiency relies in part upon destabilisation of foam bubbles, this would result in a decrease in available surface area for oxygen absorption into the fermentation medium. This in turn would give rise to a reduced decrease in dissolved oxygen concentration with increasing polymer concentration, as less oxygen would be available to the yeast for fermentation.

5.2.2.3 Pluronic L61P

Again with all levels of polymer examined, dissolved oxygen concentration fell with time.

The fall in dissolved oxygen concentration was less marked than with the fermentations with no polymer present, final dissolved oxygen levels falling to some 75% of their initial values.

Unlike Pluronic L31P, this polymer appeared to show no concentration dependence for reduction in dissolved oxygen concentration.

It is possible that as this polymer was a more effective antifoam (see section 5.2.1.3) than Pluronic L31P, at the lowest concentrations studied, the concentration dependence for reduction in dissolved oxygen concentration would be less marked.

As discussed later (see section 5.2.5.2) the presence of this polymer results in a reduction in yeast yield with increasing polymer concentration. However the fall in dissolved oxygen level was less dependant upon polymer concentration. This could suggest a paradox between the dependance of yeast yield on dissolved oxygen levels alone.

5.2.2.4 Pluronic L62P

The results obtained for dissolved oxygen levels are detailed in appendices 25 and 34. They follow a similar pattern to those obtained with Pluronic L61P. Dissolved oxygen levels only fell to 82% of starting values. There was no marked evidence for a concentration dependent relationship with dissolved oxygen level, although the smallest declines of dissolved oxygen did occur when 75 and 100 mg of polymer were present. Again the most noticeable reductions in dissolved oxygen concentration occurred during the first two hours of the fermentation when glucose availability would not be a limiting factor.

5.2.2.5 Pluronic L63P

The results obtained with Pluronic L63P followed a similar pattern to those obtained with Pluronic L31P.

There does appear to be a concentration related fall in dissolved oxygen levels. At the two lowest addition levels dissolved oxygen levels fell rapidly during the first two hours of fermentation to approximately 80% of the starting figure and continued to fall almost linearly over the next 10 hours, eventually falling to approximately 70% of the starting figure.

At the three higher addition levels, falls in dissolved oxygen levels were less marked, falling to 85% after 12 hours when 50 mg polymer was added and only 90% when 75 mg and 100 mg polymer was present.

The fall in dissolved oxygen levels with reducing polymer concentration can also be related to foam formation with higher polymer concentrations giving improved foam control.

5.2.2.6 Pluronic L64P

The trends outlined in Section 5.2.2.5 for Pluronic L63P were similar to those shown by Pluronic L64P, although differences in dissolved oxygen levels were more marked at lower polymer addition rates.

When 5 mg Pluronic L64P was added to the medium, dissolved oxygen levels fell rapidly to 80% after 2 hours and continued to fall reaching 66.5% after 12 hours. This figure is the lowest for the series of polymers containing L61P, L62P, L63P and L64P suggesting that either higher polyoxyethylene contents of the polymers results in less effect on the dissolved oxygen levels (in comparison to a system where no polymer was present), or alternatively the fact that Pluronic L64P gave very poor control of foaming at this low addition level would result in a barrier to gas release from the medium.

When 15 mg Pluronic L64P was added, again dissolved oxygen levels fell rapidly in the first two hours but only fell to a final figure of 76.5% at the end of the 12 hour fermentation.

At the three highest addition rates, the results for dissolved oxygen were similar to those for the other polymers examined. However as Pluronic L64P performed poorly as an antifoam, it is possible that the mechanism resulting in this may have been different. The fact that at these addition levels, Pluronic L64P had an adverse effect on yeast yield (see Section 5.2.5.5) may be part of the explanation. As less yeast was produced the effective demand for oxygen would have been reduced.

5.2.2.7 Pluronic L81P

This polymer showed a marked dependance on concentration for its effect on dissolved oxygen levels.

When the polymer was added at 5 mg to the medium, dissolved oxygen levels fell rapidly eventually falling to only 48% of initial readings, whereas when 15 mg of polymer was added the final figure for dissolved oxygen had only fallen to 67%. At the three highest addition levels studied, the final figures for dissolved oxygen concentration fell between 78% and 85%.

The marked difference in effect of Pluronic L81P, when added at 5 mg and 15 mg to the fermentation medium, upon dissolved oxygen levels, is in agreement with results discussed in section 5.2.1.7 relating to control of foaming. A marked improvement in control of foam was shown when the polymer addition was raised from 5 to 15 mg. Differences shown when the quantity of Pluronic L81P was increased further were less marked.

5.2.2.8 Pluronic L92P

Pluronic L92P produced similar results for dissolved oxygen concentration to Pluronic L64P and as with Pluronic L64P, the polymer was not an effective foam control agent.

In common with most of the other polymers studied, the most marked reductions in dissolved oxygen concentrations occurred with the lowest addition of polymer, 5 mg. Reductions of dissolved oxygen concentration down to only 85% of initial figures occurred with the three highest addition figures.

At the lowest addition level a rapid fall in dissolved oxygen concentration occurred over the first three hours of the fermentation in comparison to the two hours for most of the other fermentations.

5.2.2.9 Pluronic L101P

This hydrophobic polymer again showed a concentration dependent effect upon dissolved oxygen concentration, with the lowest addition of Pluronic L121P, 5 mg, resulting in the lowest dissolved oxygen concentration at the end of the fermentation period.

It is not possible to find any correlation between dissolved oxygen levels at the end of the fermentation and efficiency of antifoaming action as even at the lowest polymer concentration control of foaming was very effective. However, an inverse relationship between control of foaming and the degree to which the dissolved oxygen concentration depends upon concentration of antifoam, does appear to exist.

5.2.2.10 Pluronic L121P

Results for this polymer shown in appendices 31 and 40 are very similar to those obtained with Pluronic L101P. The only major point of difference is that for all of the addition levels studied Pluronic L121P resulted in slightly higher dissolved oxygen concentrations than those obtained with Pluronic L101P.

5.2.2.11 Summary

The concentration of dissolved oxygen in the fermentation medium fell during the course of the fermentation. This fall is associated with increased oxygen demand of the growing number of yeast cells. In the case of a fermentation where no ethylene oxide-propylene oxide copolymer was added the final dissolved oxygen levels fell to 58% of initial saturation.

Addition of ethylene oxide-propylene oxide copolymers to the medium resulted in the fall in dissolved oxygen levels being diminished. This effect was most marked at the highest addition levels studied. It was also particularly marked for the most hydrophobic polymers studied. There was some indication that the more hydrophilic polymers such as Pluronics L31P and L64P had less effect on dissolved oxygen levels than the hydrophobic polymers (when compared to the system with no polymer present).

The mechanisms associated with the changes in dissolved oxygen levels are known to be complex (72 - 74) and may well be associated with increased bubble surface area (thus increasing oxygen dissolution rate) and also with the effect of foam in the fermentation vessel headspace (the volume between the liquid surface and vessel top) on the rate of oxygen release from the medium.

5.2.3 pH

The rate of change of pH during the fermentation was recorded by means of a pH electrode connected to a pH meter (see Section 6.2.4 for description). The pH meter was also coupled to an automatic acid/base pump to enable control of pH within predefined limits.

Control of pH during the fermentation was required to enable optimum growth of Saccharomyces Cerevisiae NCYC 990. The optimum pH range for growth of this yeast is 3 - 6 (75). Thus in order to obtain high yeast yields the pH should be kept within this range. Earlier experiments carried out in this work had shown that for fermentations carried out at 30°C the optimum pH range was much narrower than reported, being 3.6 - 4.6. Thus the pH control was adjusted such that if the pH of the fermentation rose above 4.6 then 2% w/w HCl was automatically added until the pH fell below 4.6 when addition was stopped. Similarly if the pH fell below 3.65 then 2% w/w NaOH was automatically added until the pH rose above 3.65. In practice this latter point was of more relevance.

In this section, no subdivisions will be drawn detailing the effect of the ethylene oxide-propylene oxide copolymers on the rate of change of pH during the fermentation. This is because none of the polymers studied, irrespective of concentrations, appeared to have any discernable effect on rate of change of pH in comparison to a fermentation with no polymer added.

All of the fermentations were started with pH within the range 4.55 - 4.60. As soon as glucose was pumped into the fermentor the pH began to fall. In all cases studied, pH fell to 3.65 within 4 hours of the start of the fermentation. After this period 2% w/w NaOH was added to maintain pH at 3.75 $\pm$ 0.10 until the end of the fermentation.

Fall in pH during the fermentation is associated with two main factors. The factor of primary importance is carbon dioxide production by the yeast. As soon as glucose was added to the fermentation medium carbon dioxide was produced. The other factor, although of less significance,

is production of phosphoric acid. In the fermentation medium ammonium phosphate is present as an available nitrogen source. As soon as nitrogen is taken up by the yeast it will result in phosphoric acid production with subsequent fall in pH.

In addition to observing the pH in order to maintain optimum growth conditions for the yeast, it was also observed to ascertain if any of the polymers had effect on rate of pH change.

Any changes that did occur were shown to be minimal, and thus if carbon dioxide production or nitrogen assimilation was affected by the polymers, the effect was not sufficiently gross as to be observed.

However, it is assumed that carbon dioxide production will have been affected by addition of the polymers as yeast yields were affected, thus showing that some change in fermentation rate had occurred. Without access to continuous gas analysis equipment it was not possible to validate this assumption.

The results for this section are in appendices 42 - 61.

5.2.4 Temperature

The temperature throughout all of the fermentations was kept constant at 30.2 $\pm$ 0.2°C, again for optimum growth of Saccharomyces Cerevisiae NCYC 990.

During the fermentations, cell growth results in heat liberation which would increase the fermentation medium temperature above 30.2°C. Use of cooling coils, shown in Section 5.2, prevented this. No attempt was made to study the degree of cooling required in any of the fermentations in order to maintain a constant temperature of 30.2°C.

5.2.5 Yeast Yield

In fermentations with no ethylene oxide-propylene oxide copolymer present, glucose and oxygen availability were the main factors in determining yeast yield. As discussed in previous sections other conditions such as pH, temperature, availability of minerals, vitamins etc were such that high yeast yield should be obtained (76). Results discussed in section 5.2.2 showed that oxygen availability was not a limiting factor with significant quantities of dissolved oxygen being present at the end of the fermentation. It was possible that oxygen may not have been utilised if the fermentation had progressed by an anaerobic route (see section 4.2.2). However if this had been the case, in addition to no oxygen being required, significant quantities (ie greater than 0.5% w/w fermentation medium) of ethanol would have been formed. In order to rule out this possibility a sample of fermentation medium was examined immediately after the fermentation for ethanol content.

Gas liquid chromotography (77) showed an ethanol content of less than 0.1% w/w. This was also the case in fermentations with polymers present. Thus an aerobic route was followed. Therefore it was thought that glucose availability would be the major limiting factor. Analysis of the medium for glucose at the end of the fermentation (78, 79) showed that this was the case with concentrations being 0.001 gdm^{-3} or less. This was also the case for fermentations with polymer present.

Thus for a fermentation with no polymer present only glucose availability was shown to limit overall yeast yield. In this work the initial quantity of yeast added to the medium was 0.7 g (expressed as dry weight). After the 12 hour fermentation, the culture was rapidly cooled to 10°C. The yeast was then separated from the medium by centrifugation at 4000 rpm. The yeast was then washed in distilled water and again centrifuged. The weight was then recorded. A mean yeast yield of 2.388 g was produced. Thus an increase of 1.688 g dry weight occurred showing a 241% increase in cell mass.

Previous work (80) has shown that in conditions of limited glucose availability approximately 50% of available glucose would be used in increasing cell mass. This work is therefore in general agreement with that. If glucose availability was not limiting, the final yeast mass would be expected to be higher.

Part of this work was to study the effect of ethylene oxide-propylene oxide copolymers on yeast yield. Previous studies (81 - 83) had shown these type of polymers can adversely affect the growth rate of a variety of micro-organisms. However, no published data were available on their affect on yeasts. The results showing variation of yeast yield with polymer weight are shown in appendices 62 - 80. The results are discussed below.

5.2.5.1 Pluronic L31P

This polymer caused reduction in yeast yield at all polymer weights studied. This fact was common for all of the polymers studied. The most dramatic reductions, 62% in yeast yield occurred when 50 mg or more, of polymer, was added to the fermentation medium. Reductions in yeast yield for the two lowest addition levels studied were less marked but even in the presence of only 5 mg Pluronic L31P, a fall in yeast yield of some 38% was observed.

Earlier studies (84) have shown that both ethylene oxide-propylene oxide copolymers and propylene oxide homopolymers with molecular weight below 2000 can be toxic to a number of organisms.

Pluronic L31P has a weight average molecular weight of 974 and was the only polymer studied with a molecular weight below 2000. It appears to have been toxic to Saccharomyces Cerevisiae NCYC 990.

A number of possible mechanisms could be used to explain the reduction in yield. Polymers of this type are degraded by attack of the terminal hydroxyl groups (85). The lower the molecular weight polymer would be more readily degraded having less polyoxypropylene and polyoxyethylene

units per molecule than the higher molecular weight anologues. Attack of the hydroxyl group will result in the formation of short chain alcohols and subsequently aldehydes. A number of short chain alcohols and aldehydes are toxic to yeasts (86) and this may explain why yeast yields were lowered in the presence of Pluronic L31P, as lower molecular weight polymers will have more terminal groups per gram than higher molecular weight polymers. If degradation occurs via end groups, then it is most likely to occur with lower molecular weight polymers.

An alternative explanation may rely on the polymer being adsorbed on to the yeast cell surface. Results in section 5.3 show that over 10% of the polymer added to the fermentation is either adsorbed on to the cell surface or taken into the cell. Presence of the polymer on the cell surface would act as a partial barrier to oxygen transport across the cell wall. Results in section 5.2.2 do suggest less oxygen is utilised during the fermentation when polymer levels are 50 mg or higher. With limited oxygen use it would be anticipated that cell growth would be diminished, with more of the available energy derived from glucose being used in cell maintenance. Under conditions of limited oxygen availability glucose may be converted to glycogen for storage (87).

Thus two differing mechanisms could be operating in reducing yeast yield. One relates to the polymer limiting oxygen availability to the cell, the other suggests that either the ethylene oxide-propylene oxide or its degredation products are toxic to the yeast resulting in a change in cell activity.

5.2.5.2 Pluronic L61P

Again the presence of this polymer in the fermentation medium resulted in a reduction in yeast yield. The most significant reduction in yeast yield, 41%, occurred when the addition level of polymer was highest. At the lowest level of polymer addition, 5 mg, only a 6% reduction in yeast yield occurred. At all levels examined this polymer had less adverse affects on yeast yield than did Pluronic L31.

The weight average molecular weight of Pluronic L61P is 2271, above the figure where previous work has shown these polymer types to be toxic.

5.2.5.3 Pluronic L62P

This polymer had less adverse effect on yeast yield when present at 100 mg in the fermenation medium causing only a 31% fall in yield. However this reduction in yield was also apparent when only 15 mg polymer was present. It was only at the lowest addition level, 5 mg, that reduction in yeast yield was smaller, ie. 18%.

Pluronic L62P appeared to have a more adverse effect on yeast yield than Pluronic L61P at all addition levels studied, with the exception of the 100 mg addition. The major composition difference between Pluronic L61P and L62P is that the latter has a larger polyoxyethylene content. This fact may be associated with increased toxicity to the yeast as it would be more likely that the yeast cell could cause cleavage of polyoxyethylene units from the polymer than polyoxypropylene units.

In addition Pluronic L62P was a less effective foam control agent and it is known (88) that when foam is present the yeast can stabilise the foam, effectively removing the cells from the medium, thus starving them of oxygen and glucose. This is thought likely to be in part responsible for the reductions in yield observed.

5.2.5.4. Pluronic L63P

When added at levels of 50 mg and above this polymer caused as much as 57% reduction in yeast yield. However unlike the polymers previously discussed it also caused large reductions in yeast yield at low addition rates. When only 5 mg Pluronic L63P was present, a 39% fall in yeast yield occurred.

It is thought unlikely that Pluronic L63P would have been directly toxic to the yeast as all of the polymer remained in the fermentation medium throughout the fermentation (see section 5.3). Thus none of the polymer could have been adsorbed on to the cell surface.

The reduction in yeast yield is more likely to have occurred as a result of the polymer affecting dissolved oxygen concentration or due to the yeast being taken out of the fermentation medium on stabilised foam.

5.2.5.5 Pluronic L64P

A more gradual fall in yeast yield with increasing polymer concentration occurred. Reductions in yeast yield increased from 23% when 5 mg polymer was present to 57% when 100 mg was present.

The reasons for reduction in yield are thought likely to be similar to those for Pluronic L63P.

5.2.5.6 Pluronic L81P

Yeast yield fell with increasing polymer weight. At the lowest addition level a reduction of 23% was observed but at the highest addition level a reduction of 53% occurred.

It is thought unlikely that the reductions were as a result of yeast being removed from the fermentation medium by stabilised foam as the polymer controlled foaming well, particularly at the higher addition levels. It is more likely that the reductions are associated with the toxicity of the polymer or its degradation products or interference of the polymer with gas transport. As much as 10% of added polymer was either adsorbed onto the surface of the yeast and/or metabolised by it during the fermentation (see section 5.3).

5.2.5.7 Pluronic L92P

The effect of varying addition levels on yeast yield for this polymer showed less concentration dependence than the polymers discussed in Section 5.2.5.1 and Section 5.2.5.6. Reductions in yeast yield varied from 31% to 47%.

In the case of Pluronic L92P both the toxicity of the polymer and its poor control of foaming may play a part in reducing yeast yield.

5.2.5.8 Pluronic L101P

This polymer showed very little concentration dependent effect on yeast yield. Addition of 5 mg polymer resulted in a sharp reduction, 34%, in yield but even at the highest addition level only a 45% reduction in yield occurred.

As control of foaming by Pluronic L101P was excellent, its adsorption and toxicity to the yeast is thought to be a factor in reducing yield. Also section 5.3 shows that as much as 16% of added polymer was adsorbed onto the yeast surface which could reduce oxygen transport across the cell wall. Far more of this hydrophobic polymer was adsorbed than any of the others so far discussed.

5.2.5.9 Pluronic L121P

This polymer behaved in a similar manner to Pluronic L101P with respect to its effect on yeast yield. It caused a 41% reduction in yield when added at 5 mg to the fermentation medium but higher levels of polymer addition had little further effect. When 100 mg polymer was added a reduction of 49% occurred.

The reasons for the reduction in yeast yield are likely to be similar to those for Pluronic L101P. Pluronic L121P was the most hydrophobic polymer studied and it was the most heavily adsorbed onto the yeast with over 22% being adsorbed when 100 mg was added to the fermentation medium. Thus any effect on oxygen adsorption will have been increased.

5.2.5.10 Summary

All of the ethylene oxide-propylene oxide copolymers examined caused a reduction in yeast yield.

A number of factors may be involved in this effect and are likely to be associated with the toxicity of the polymers and their degradation products. Pluronic L31P, the lowest molecular weight polymer studied, was found to reduce yeast yield as expected from previous studies.

The reduction of yeast yield was found to be less concentration dependent for the two highest molecular weight polymers.

There was some indication that for a polyoxypropylene base of set molecular weight, increasing polyoxyethylene content resulted in a reduction in yeast yield.

Polymers showing poor foam control may result in lowering of yeast yield due to loss of yeast from the fermentation medium on stabilised foam.

Adsorption of the polymers onto the yeast cell surface may also act as a barrier to oxygen adsorption.

The presence of 5 mg Pluronic L61P in the fermentation medium had relativly little effect upon yeast yield.

5.3 Detection of Ethylene Oxide-Propylene Oxide Copolymers

Ethylene oxide-propylene oxide copolymers are used in yeast fermentations to inhibit foam formation. However, as has been discussed in section 5.2, these polymers can adversely affect yeast yield. In order to ascertain whether the polymers were reducing yeast yield as a result of their toxicity to Saccharomyces Cerevisiae NCYC 990, or by other mechanisms, it was necessary to determine how much polymer had been adsorbed onto the yeast. In view of the complexity of the chemistry of yeast cell membranes it was decided to determine the quantity of polymer remaining in the fermentation medium. As subsequent work showed that none of the polymer was adsorbed onto the culture vessel walls or onto the surfaces of any probes immersed in the medium, it was assumed that any polymer "lost" from the fermentation medium had been adsorbed onto the yeast cells.

A number of techniques were evaluated for determination of the polymer concentration (89-96). Initially work was centered upon the effect of the polymers on the surface tension of the fermentation medium. However as can be seen from the following table changes in surface tension caused by changes in polymer concentration (of unpurified polymer) were small.

POLYMER	SURFACE TENSION (dyne cm^{-1}) OF MEDIUM USING FOLLOWING POLYMER CONCENTRATION w/w %				
	0.10	0.05	0.03	0.01	0.005
Pluronic L61	42.6	43.8	44.8	47.2	47.8
Pluronic L62	41.0	43.3	44.2	45.6	47.2
Pluronic L64	41.3	44.2	45.6	47.2	48.7
Pluronic F68	51.4	53.2	54.0	55.1	55.8

For each polymer studied the changes in surface tension were not sufficiently large to be of use in quantitative work.

Thus a number of chemical techniques were evaluated for determining the concentration of polymers. These fall into 3 separate groups:-

5.3.1

Pyrolysis of the polymer followed by subsequent determination of acetaldehyde and propionaldehyde either colourimetrically or chromatagraphically. The literature suggests this method has poor reproducibility, giving ambiguous quantitative results. This latter point appeared true for the ethylene oxide-propylene oxide polymers examined in the work. The technique was therefore abandoned.

5.3.2

Ethoxylated materials are said to result in the liberation of stoichiometric quantities of iodine from HI under reflux. A general scheme being:

$$(CH_2CH_2O)x + 2xHI \longrightarrow x\ ICH_2CH_2I + xH_2O$$

$$ICH_2CH_2I \xrightarrow{\Delta} CH_2 = CH_2 + I_2$$

or $$ICH_2CH_2I + HI \longrightarrow CH_3CH_2I + I_2$$

Determination of liberated iodine should have enabled quantitative determination of the ethoxylated material. Unfortunately, the technique was not found to be reproducible for the ethoxylated polymers studied in this work.

5.3.3

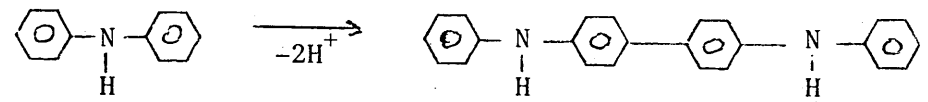
A number of complexiметric methods (97-100) have been reported for the determination of the concentration of ethylene oxide adducts. However modification of a method reported by Schoenfeldt gave quantitative results for all of the polymers studied.

The method relies on complex formation between the ethylene oxide-propylene oxide polymer and potassium ferrocyanide. Although a definitive mechanism is not known for the complex formation it is thought to take place between ether oxygen atoms in the ethylene oxide-propylene oxide subunits and the ferrocyanide. This assumption is based on previous findings by Von Baeyer and Villiger (101) that ferrocyanic acid gives addition products with diethyl ether. The complex is thought to contain oxonium ions (102).

Although the method is described in detail in 6.4 it involves determination of residual ferrocyanide using zinc sulphate as follows:-

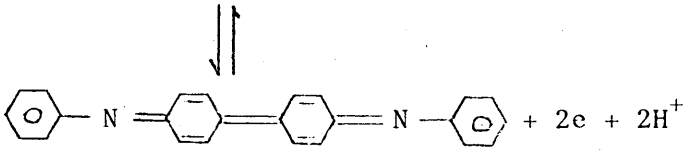
$$3Zn^{2+} + 2K_4\left[Fe(CN)_6\right] \longrightarrow K_2Zn_3\left[Fe(CN)_6\right]_2 + 6K^+$$

The end point of the titration was followed using diphenylamine as a redox indicator as shown below.



Diphenylamine

Diphenyl Benzidine



Diphenyl Benzidine violet

The end point was characterised by a change in solution from almost colourless to pale green and then sharply to a deep blue violet.

The technique was examined using all of the purified polymers. In each case a direct relationship was found between weight of ethylene oxide-propylene oxide polymer and number of moles of ferrocyanide complexed. Graphs for each of the polymers examined are shown in appendices 81-89. It was noticed that more ferrocyanide was complexed with polymers with higher polyoxyethylene content possibly suggesting partial steric hindrance to complex formation associated with the methyl group in propylene oxide.

The method was used to determine residual polymer in the fermentation medium after the 12 hour fermentation period was complete. After centrifugation of the medium to remove yeast, a 100 cm^3 aliquot was taken and the concentration of polymer determined using the graphs and tables shown in appendices 81-98. In the cases of the most hydrophobic polymers used at the lowest levels (0.005 g in the original medium) it was necessary to use a 250 cm^3 aliquot in order to obtain an accurate figure for the quantity of polymer remaining in the aqueous phase.

The results obtained are detailed below, each result representing the mean of three determinations for each fermentation.

5.3.3.1 Pluronic L31P

The weight of polymer remaining in the fermentation medium for each concentration studied is shown below.

INITIAL POLYMER WEIGHT /g	POLYMER WEIGHT IN FERMENTATION MEDIUM AFTER 12 HOURS /g	PERCENTAGE OF POLYMER ADSORBED ONTO YEAST
0.005	0.0044	12.0
0.015	0.0131	12.7
0.050	0.0437	12.6
0.075	0.0642	14.4
0.100	0.0857	14.3

These results show that for Pluronic L31P the majority of the polymer remains in the aqueous phase. This result is not perhaps surprising as the polymer has a cloud point of 32.5°C suggesting that it should be essentially soluble in the fermentation medium at 30°C. However, as was described in the section covering polymer characterisation, each of the ethylene oxide-propylene oxide copolymers cannot be considered to be discrete entities but are a number of species of differing molecular weights. Hence although the polymer, Pluronic L31P, is essentially water soluble, it may well be the more hydrophobic fractions that are "lost" from the aqueous phase being adsorbed by the yeast.

No evidence is available in this work to suggest when the polymer was adsorbed onto the yeast cell surface. However it is assumed that any loss of polymer would occur soon after yeast was introduced ie. as soon as a hydrophobic surface was introduced. Adsorption of polymer onto the yeast at the start of the fermentation would have more effect than if adsorption occurred later. As has been postulated earlier, adsorption of the polymer onto the cell surface could have two effects. One would be to act as a barrier to oxygen uptake and secondly if the polymer was metabolised by the cell, degradation products could be toxic. At the start of the fermentation yeast cell concentration and also total cell surface area would be at their lowest and hence adsorption of polymer at this stage would result in a larger surface area per organism covered and the possibility of higher concentrations of toxins forming.

Section 5.2 shows that use of Pluronic L31P does result in a reduction in yeast yield. The fact that over 12% of the weight of polymer used is adsorbed by the yeast may contribute to this. As Pluronic L31P was the lowest molecular weight polymer examined it is more likely to be absorbed into the cell rather than remain on the surface. Previous data for non-polymeric species has shown that molecular weight is a limiting factor on rate of absorption into the cell (82).

5.3.3.2 Pluronic L61P

INITIAL POLYMER WEIGHT / g	POLYMER WEIGHT IN FERMENTATION MEDIUM AFTER 12 HOURS / g	PERCENTAGE OF POLYMER ADSORBED ONTO YEAST
0.005	0.0041	18.0
0.015	0.0122	18.7
0.050	0.0412	17.6
0.075	0.0631	15.9
0.100	0.0840	16.0

This polymer is more hydrophobic than Pluronic L31P and not surprisingly more is adsorbed onto the yeast cell surface, which is essentially hydrophobic. Pluronic L61P is of higher molecular weight than Pluronic L31P and would be less readily absorbed into and broken down by the cell. Loss of polymer from the aqueous phase may also have a detrimental effect on the fermentation in as much as the concentration of polymer available to migrate to a gas/liquid interface is effectively reduced. In the case of this particular polymer as much as 18.7% is removed from the aqueous phase.

5.3.3.3 Pluronic L62P

INITIAL POLYMER WEIGHT / g	POLYMER WEIGHT IN FERMENTATION MEDIUM AFTER 12 HOURS / g	PERCENTAGE OF POLYMER ADSORBED ONTO YEAST
0.005	0.0047	6.0
0.015	0.0139	7.3
0.050	0.0461	7.8
0.075	0.0696	7.2
0.100	0.0932	6.8

This polymer is based on the same polyoxypropylene polymer as Pluronic L61P but has a higher polyoxyethylene content and is thus more hydrophilic. These facts are reflected in the quantity of polymer adsorbed onto the yeast. Only some 7% is removed from the fermentation medium. Thus at the lowest concentration studied only 0.3 mg Pluronic L62P is adsorbed onto an initial dry yeast mass of 700 mg (actual yeast mass (wet) was 8900 mg). This may explain why this particular polymer had a relatively limited effect on yeast yield when only 5 mg polymer was added to the fermentation medium.

5.3.3.4 Pluronic L63P and Pluronic L64P

Detection of these two polymers in the fermentation medium after a 12 hour fermentation showed that, at all of the concentrations studied, 100% of the polymer remained in the aqueous phase with none being adsorbed onto the yeast. It is therefore thought unlikely that toxicity of the polymers to the yeast would have been a major contributory factor in the reduction in yeast yields that occurred when these polymers were present.

The fact that 100% Pluronic L64P remained in the aqueous phase is to be expected as the polymer is markedly more hydrophilic than those previously discussed, having an ethylene oxide content of 38.9% and a cloud point of 37.6°C in the fermentation medium.

The results for Pluronic L63P are more surprising. This polymer was essentially insoluble in the fermentation medium having a cloud point of 25.8°C. Also it only contains slightly more ethylene oxide than Pluronic L62P, 26.1% compared to 23.5%. It would have been expected that some (less than 5%) of the polymer would have been removed from the medium in view of the results obtained for Pluronic L62P.

5.3.3.5 Pluronic L81P

INITIAL POLYMER WEIGHT / g	POLYMER WEIGHT IN FERMENTATION MEDIUM AFTER 12 HOURS / g	PERCENTAGE OF POLYMER ADSORBED ONTO YEAST
0.005	0.0046	8.0
0.015	0.0138	8.0
0.050	0.0460	8.0
0.075	0.0669	10.8
0.100	0.0907	9.3

These results are as expected for a hydrophobic polymer that was essentially insoluble in the fermentation medium. Again the fact that only 0.4 mg polymer was adsorbed onto the yeast at the lowest polymer weight studied may explain why only a limited reduction in yeast yield occurred. However when 100 mg polymer was added to the initial fermentation medium, the amount of polymer adsorbed increased to 9.3 mg ie. more than 23 times as much as was the case of the lowest addition level. The greatest reduction in yeast yield occurred when 9.3 mg polymer was adsorbed onto the yeast. In fact this quantity of polymer represented almost 1% of dry yeast weight.

5.3.3.6 Pluronic L92P

INITIAL POLYMER WEIGHT / g	POLYMER WEIGHT IN FERMENTATION MEDIUM AFTER 12 HOURS / g	PERCENTAGE OF POLYMER ADSORBED ONTO YEAST
0.005	0.0049	2.0
0.015	0.0146	2.7
0.050	0.0483	3.4
0.075	0.0724	3.5
0.100	0.0971	2.9

The above results show that only small quantities of Pluronic L92P are lost from the aqeuous phase during fermentation. The results are similar to those for Pluronic L62P which has a similar ethylene oxide content. Pluronic L92P has a higher cloud point than Pluronic L62P which would suggest that more of the polymer should remain in the aqueous phase.

The fact that only approximately 3% of the polymer weight is adsorbed onto the yeast is thought unlikely to explain the reductions in yeast yield obtained when this polymer was present in the fermentation medium.

5.3.3.7 Pluronic L101P

INITIAL POLYMER WEIGHT / g	POLYMER WEIGHT IN FERMENTATION MEDIUM AFTER 12 HOURS / g	PERCENTAGE OF POLYMER ADSORBED ONTO YEAST
0.005	0.0043	14.0
0.015	0.0130	13.3
0.050	0.0427	14.6
0.075	0.0631	15.9
0.100	0.0851	14.9

Pluronic L101P is a hydrophobic polymer containing only 12.9% ethylene oxide and as expected larger quantities of polymer were adsorbed onto the yeast than was the case with the more hydrophilic polymers studied. The results obtained are similar to those found with Pluronics L31P and L61P. At the highest concentrations studied 14.9 mg of Pluronic L101P was adsorbed onto the yeast. This was greater than 1.1% of the weight of dry yeast produced. It seems likely that adsorption of this polymer onto the yeast may well influence yeast yield.

5.3.3.8 Pluronic L121P

INITIAL POLYMER WEIGHT / g	POLYMER WEIGHT IN FERMENTATION MEDIUM AFTER 12 HOURS / g	PERCENTAGE OF POLYMER ADSORBED ONTO YEAST
0.005	0.0041	18.0
0.015	0.0122	18.7
0.050	0.0408	18.4
0.075	0.0600	20.0
0.100	0.0794	20.6

These results show that Pluronic L121P was adsorbed more strongly onto the yeast than any of the other polymers studied. It gave rise to similar results to those found with the other hydrophobic polymers studied.

At the highest concentration examined 20.6 mg polymer was adsorbed onto the yeast representing almost 1.7% of dry yeast weight. As with Pluronic L101P it is thought likely that polymer adsorption is at least partly responsible for reduction in yeast yield.

5.3.3.9 Summary

A modification of a method developed by Schoenfeldt was used to determine the quantity of residual ethylene oxide-propylene oxide copolymer in the fermentation medium.

The results suggest that the adsorption of polymer onto the yeast is related to the hydrophobicity of the particular polymer. High molecular weight hydrophobic polymers appear to be particularly strongly adsorbed. It is thought that this latter fact may be in part an explanation for reductions in yeast yield associated with the use of these polymers.

5.4 Summary of Results of Fermentation Parameters

A number of ethylene oxide-propylene oxide copolymers have been examined for their effect upon a number of fermentation parameters, in particular the fermenter contents volume, dissolved oxygen concentration, pH and yeast yield.

One of the aims of the work was to ascertain if any polymers were capable of controlling foam formation in the fermentation vessel without adversely affecting yeast yield.

Some of the general trends observed are detailed below.

a) Increasing polyoxyethylene content resulted in a reduction in effectiveness of the polymers with respect to foam control. Polymers with lower polyoxyethylene contents (10.9 - 14.6%) showed an increase in effectiveness with increasing molecular weight.

b) All of the polymers examined adversely effected the rate of fall of dissolved oxygen concentration. The effect became more pronounced with increasing polymer weight.

c) None of the polymers studied appeared to affect the rate of change of pH during the fermentation.

d) All of the polymers caused a reduction in yeast yield. This effect was concentration dependent with greatest reductions occurring with highest polymer weights.

Of the polymers studied, Pluronic L61P had least effect upon yeast yield yet still was capable of controlling foam formation.

Chapter 6

EXPERIMENTAL

6.1 Cultures of Saccharomyces Cerevisiae NCYC 990

6.1.1 Pre-Cultures

The specific strain of Saccharomyces Cerevisiae was obtained from The National Collection of Yeast Cultures (103). It is a general strain that was first identified by Chivas Bros. Ltd in 1981. The culture is maintained by freeze drying and individual cultures are supplied in sterile ampoules. Each ampoule contains ca. 10 mg freeze dried yeast and a cotton wool plug for maintenance of sterility.

The yeast was recovered from the freeze-dried culture as follows.

The ampoule was wiped with ethanol to ensure sterility. A file cut was made on the ampoule mid way along the cotton wool plug. The file mark was touched with red hot glass to crack the ampoule and the cotton wool plug removed. Difco YM broth (0.5 cm^3) was added aseptically. The contents of the ampoule were mixed well and transferred to a conical flask (250 cm^3) containing YM broth (200 cm^3). The culture was incubated for 7 days at 28°C. After this period part of the culture (1.0 cm^3) was aseptically transferred to further flasks containing YM broth. The operation was repeated every 7 days to maintain a constant source of yeast.

Difco YM broth has the following composition:

Bacteriological Yeast Extract	:	3 gdm^{-3}
Malt Extract	:	3 gdm^{-3}
Bacteriological Peptone	:	5 gdm^{-3}
Bacteriological Dextrose	:	10 gdm^{-3}

The broth was prepared by dissolving Difco YM powder (21 g) in distilled water (1000 cm^3) and sterilising the solution at 121°C. The broth was cooled to 28°C prior to use.

After culturing it in this broth the yeast was separated by centrifugation for use in the fermentation studies. Aliquots (50 cm^3) of the culture were aseptically transferred to sterile centrifuge tubes, and centrifuged at 4000 rpm for 10 minutes. The broth was decanted and sterile distilled water (25 cm^3) was added to the yeast and mixed with it. The suspension was centrifuged again at 4,000 rpm for 10 minutes. The water was decanted off. The yeast was then aseptically transferred to a sterile sample jar (10 cm^3 capacity). The yeast at this stage was in the form of a paste with a non-volatile content of 20 - 22% (determined by drying at 130°C for 30 minutes).

The equivalent of 0.7 g (dry weight) of yeast was aseptically transferred to a further sample jar and sterile distilled water (2 cm^3) was added to produce a mobile yeast suspension that could be readily transferred to the fermentation vessel.

6.1.2 Medium for Fermentation Studies

The medium used throughout the fermentation studies had the following composition.

D-Biotin	:	0.1 mg
$MgCl_2 . 6H_2O$ (Analar)	:	0.15 g
$MgSO_4 . 7H_2O$ (Analar)	:	0.05 g
Pantothenic Acid (Ca^{2+} salt)	:	0.01 mg
Aneurine Hydrochloride	:	1.0 mg
Cocarboxylase	:	1.0 mg
NH_4OH (0.88)	:	3.0 cm^3
H_3PO_4 (85% w/w GPR)	:	0.25 cm^3
Distilled water	:	to 1000 cm^3

The magnesium chloride, magnesium sulphate and ammonium hydroxide were dissolved in water (ca. 200 cm^3). The phosphoric acid was added and dissolved followed by pantothenic acid, aneurine hydrochloride, cocarboxylase and D-biotin. The pH of the medium was adjusted to 4.3 with 25% w/w sulphuric acid. The medium was then diluted to 1000 cm^3 with distilled water and sterilised at 121°C for 30 minutes.

After cooling 500 cm^3 of the medium was aseptically transferred to the sterile fermentation vessel. D-glucose solution (3 cm^3) was then added.

The yeast was then added. D-glucose (50 gdm^{-3}) was then added for 12 hours at a rate of 6 cm^3 hr^{-1}. The D-glucose solution had been sterilised at 121°C for 15 minutes.

During the course of the fermentation sodium hydroxide (25 gdm^{-3}) was added to maintain pH within previously described limits. As vapours formed during sterilisation of sodium hydroxide solutions could result in damage to the autoclave, the solution was made by dissolving sodium hydroxide (AnalaR) pellets in sterile water. The base is considered sterile by virtue of its strength (104).

6.2 Fermentation Apparatus

A schematic representation of the fermentation apparatus was given in Section 5.2. A more detailed description is given below. (Also see references 105 - 114)

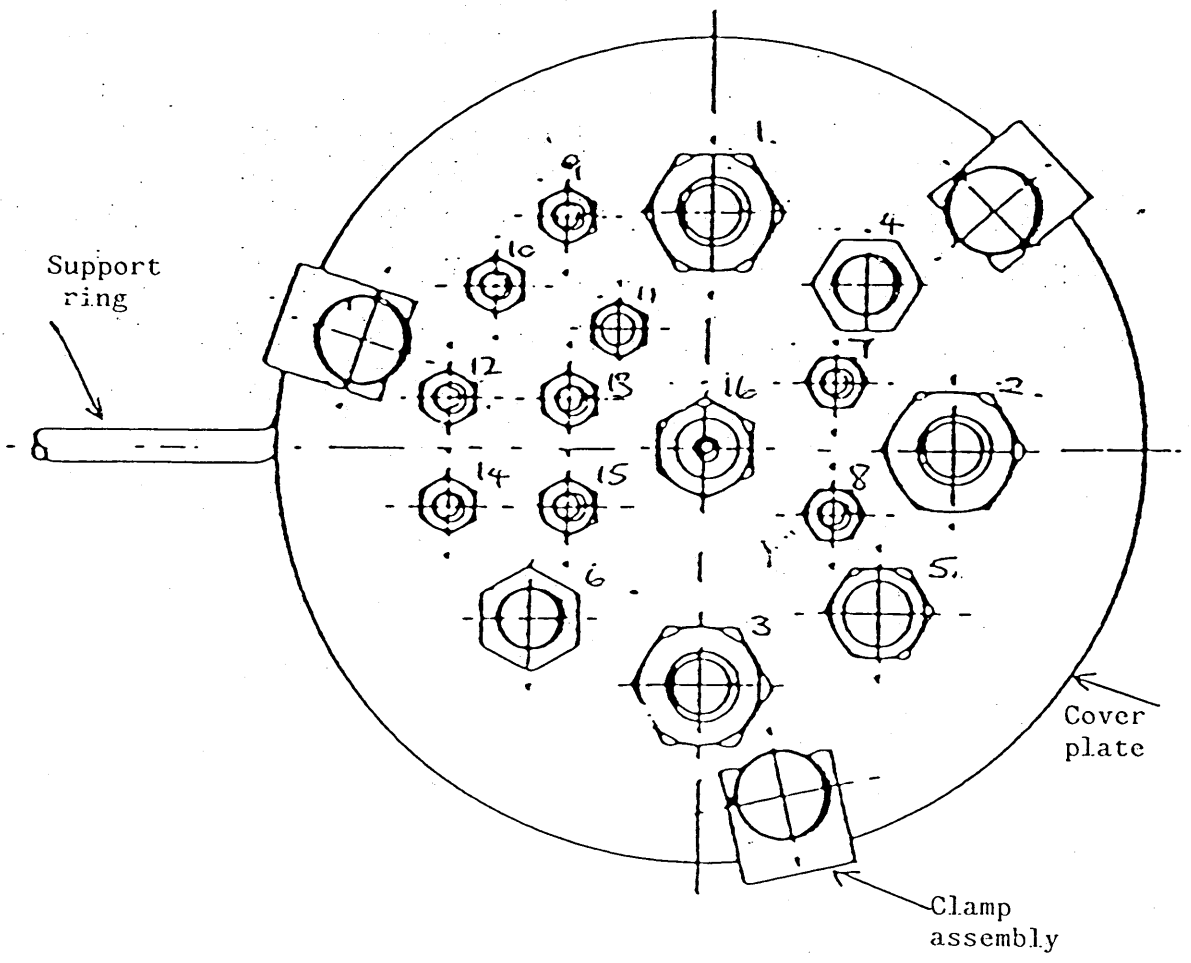
The fermentation vessel was a cylindrical flat bottomed borosilicate glass vessel with a 100 mm bore ground flat flange. It had a height of 165 mm and diameter of 105 mm with a gross capacity of 1300 cm^3.

A cover plate with 16 access ports was clamped onto the vessel by three clamps attached to a toroidal ring, which was compressed between the vessel flange and cover plate.

The access ports were of varying diameter and were used so that various probes could be introduced into the fermenter as follows:

PORT NUMBER	BORE (mm)	USE OF PORT
1	12.5	pH electrode
2	12.5	oxygen electrode
3	12.5	-
4	9.5	thermometer
5	9.5	condenser
6	9.5	thermister
7	5.0	acid entry
8	5.0	base entry
9	5.0	glucose entry
10	5.0	cooling coil
11	5.0	cooling coil
12	5.0	air entry
13	5.0	thermister
14	5.0	-
15	5.0	-
16	5.0	Central stirrer shaft

The numbered entry ports are shown below.

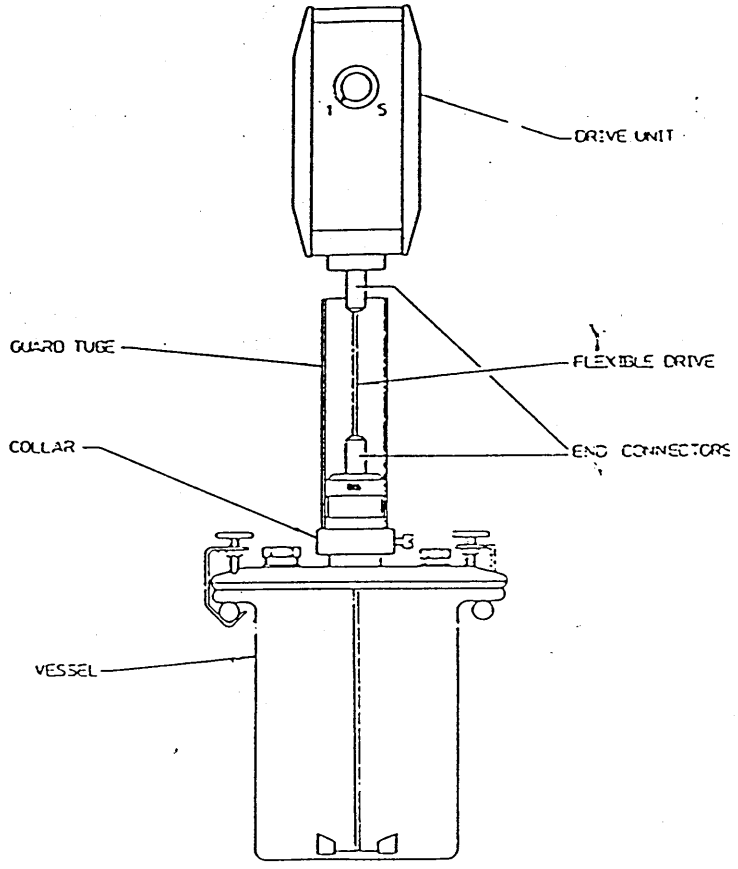


O rings compressed by gland nuts formed airtight seals between the ports and components entering the vessel. Any ports not used were sealed off.

Components were introduced into the vessel such that the stirrer blade, cooling coil and air entry tube were 5 mm above the base of the container. All the other components were positioned 30 mm above the container base.

The sealed vessel and contents were sterilised at 121°C for 30 minutes prior to each fermentation.

Agitation of the fermenter contents was carried out by a direct drive stirrer capable of providing a variable speed from 200 to 1200 rpm. The assembly is shown below.



Throughout the course of this work a stirrer speed of 600 rpm was used. This speed was periodically checked to ensure speed variation was within 1% of 600 rpm. A speed of 600 rpm was chosen as it produced good vortex aeration whilst still allowing easy recording of liquid levels. Higher stirrer speeds caused great fluctuations in liquid levels.

6.2.1 Temperature Control

Temperature in the fermentation medium was maintained at 30.2 $\pm$ 0.2°C by means of a solid state thermostat, thermister sensing probe, immersion heater and cooling coil.

The thermister, heater and cooling coil were fitted into the cover plate as outlined in section 6.2.

Cooling was required intermittently during the fermentation and was brought about by water flow through the cooling coil. This was controlled by a solenoid valve controller which opened automatically, actuated by a control compensator when cooling was required. Combined with the proportional action of the thermostat this produced automatic temperature control with little fluctuation or drift.

All of the components for temperature control were sterilised at 121°C prior to use in the fermentation vessel with all electrical contacts being covered with aluminium foil during sterilisation for protection.

6.2.2. Fermenter Contents Volume

The fermenter contents volume was recorded visually throughout the fermentation. The fermentation vessel had been graduated prior to the start of fermentation. In all cases the combined volume of liquid and foam were recorded up to a maximum of 1000 cm^3. Although the vessel had a gross capacity of 1300 cm^3 no values greater than 1000 cm^3 were recorded due to unreliability of these results because of potential loss of vessel contents via the air condenser.

At the start of the fermentation, medium (500 cm^3) and D-glucose solution (3 cm^3) was introduced into the vessel. This combined with the volume occupied by probes and the stirrer gave a total initial volume of 550 cm^3.

In addition to increase in volume as a result of foaming and aeration, the volume of vessel contents was increased as a result of continual addition of D-glucose (50 gdm^{-3}) at a rate of 6 cm^3 hr^{-1}. Minor increase in volume occurred due to addition of sodium hydroxide solution to maintain pH above 3.65. However this volume was only approximately 2 cm^3.

The D-glucose was added by means of a peristaltic pump. The addition rate was frequently monitored by means of a 3 way changeover clamp and Y connector, connected to a graduated pipette (10 cm^3). Silicone rubber tubing (1 to 8 mm bore) was used for all connections from the D-glucose storage vessel to the fermentation vessel. All tubing and pipettes were sterilised by autoclaving them at 121°C. Cotton wool plugs were used on the storage vessel and pipette to maintain sterility.

As discussed in Section 5.2.1 the presence of ethylene oxide-propylene oxide copolymers had a significant effect upon the volume of the fermentation vessel contents. The polymers were sterilised (dry) at 130°C for 1 hour. They were added aseptically to the fermentation vessel prior to addition of the fermentation medium.

Readings of the vessel contents volume were made throughout the fermentation. They were made by stopping the stirrer, recording the volume (whilst maintaining aeration) and immediately restarting the stirrer. This process took less than 3 seconds and did not allow time for any collapse of foam to occur.

6.2.3 Dissolved Oxygen

Air was introduced into the fermentation medium by two means; vortex aeration accomplished by air in the space above the culture being drawn down and dispersed by the stirrer turbine and secondly by sparging.

The air for sparging was supplied by a twin compressor capable of providing a maximum of 5000 cm^3 min^{-1}. Air flow to the culture vessel was adjusted by means of a rotating float flowmeter set at 500 cm^3 min^{-1}. Air from the flowmeter passed through two bacteriological air filters. These had been previously sterilised and each had a minimum retention of 99.95% for particles of 0.3 μm. Air passed through the filters and was introduced into the medium through a 200 mm x 5 mm stainless steel tube positioned as shown in Section 6.2.

A water cooled outlet condenser removed water vapour from the air leaving the vessel. Use of the condenser was essential as it de-saturated the air leaving the vessel before it reached a further bacteriological filter. If condensation had occurred in the filter, contamination could have occurred leading to backgrowth into the vessel.

The dissolved oxygen concentration in the medium was determined by means of an oxygen sensitive electrode attached to an oxygen meter.

The electrode used was of the Mackereth type, consisting of a perforated silver cathode and a lead anode immersed in an alkaline electrolyte (110 gdm^{-3} $KHCO_3$ and 35 gdm^{-3} Na_2CO_3 in distilled water). The electrode was covered by a poly(tetrafluoroethylene) membrane which is selectively permeable to oxygen. The membrane was used to prevent fouling of the electrode.

The electrode, which was sterilised at 121°C prior to use, was introduced into the vessel as described in Section 6.2.

The meter has two ranges of 0 - 20% and 0 - 100% where 100% corresponds to the saturated oxygen content of the medium at a particular temperature. Prior to use in the fermentation the meter had to be calibrated.

Calibration was carried out as follows:

The fermenter was assembled as shown in Section 6.2. The stirrer was adjusted to 600 rpm and the temperature of the fermentation medium set at 30.2°C. A low pressure, oxygen free, nitrogen supply was connected in place of the aerator. Nitrogen was injected at 1000 cm^3 min^{-1} until the oxygen meter, set to the 100% range, could be adjusted to read zero and remained on zero for at least a 10 minute test period. The nitrogen supply was then disconnected and air injected at 500 $cm^3 \cdot min^{-1}$ until the oxygen meter could be set to read 100% and remained there for at least a 10 minute test period.

This procedure was repeated before each fermentation to overcome any variations occurring due to sterilisation or ageing of the electrode and membrane. The electrode was thoroughly washed after each fermentation to remove any yeast cells.

6.2.4 pH

pH during the fermentations was recorded by means of a combination pH electrode connected to a pH meter incorporated into a pH controller.

Prior to each fermentation the electrode was sterilised at 121°C. It was then immersed in the medium at 30.2°C after calibration using sterile buffers of pH 4, 7 and 9.

The pH of the medium was maintained above pH 3.65 and below pH 4.60 throughout the course of the fermentation as follows:

The indicating pH controller had a 95 mm long scale graduated from 0 to 14 pH. It incorported two set points which were independently adjustable over the whole range. In this case the points were set at pH 3.65 and 4.60. If the pH moved outside of either of these points one of the two built in peristaltic pumps was activated to deliver either 2% w/w sodium hydroxide or hydrochloric acid into the vessel until the pH returned within the limits. All pump tubing and connections were sterilised at 121°C prior to each fermentation.

At the end of each fermentation the pH electrode was cleaned in distilled water to minimise any fouling effect the yeast had upon the electrode membrane.

6.3 Yeast Yield

At the end of each fermentation, the cover plate and probes were separated from the culture vessel. The culture was then aseptically transferred to a sterile 1000 cm^3 flask. The culture vessel and probes were then carefully rinsed in sterile distilled water (25 cm^3) to recover any yeast fouling probes, stirrers etc. This was aseptically transferred to the flask combining the culture to provide a total volume of 600 cm^3.

Aliquots of the culture (50 cm^3) were aseptically transferred to centrifuge tubes and centrifuged (4000 rpm for 10 minutes). The medium was decanted off and retained for determination of ethylene oxide-propylene oxide polymer content.

To the remaining yeast paste, sterile distilled water (25 cm^3) was added. The yeast was dispersed in the water and the dispersion centrifuged (4000 rpm for 10 minutes).

The yeast paste was aseptically transferred to a pre-weighed sterile glass ampoule. The weight of yeast paste was recorded.

Yeast paste (approximately 1 g) was accurately weighed onto a pre-weighed aluminium foil dish and dried to constant weight at 130°C. This enabled the non-volatile content and hence dry yeast weight to be calculated.

6.4 Detection of Ethylene Oxide-Propylene Oxide Copolymer

The method described is a slight modification of that described by Schoenfeldt.

Reagents

1. 0.25 M Potassium ferrocyanide (AnalaR) dissolved in 0.05% w/v anhydrous sodium carbonate (AnalaR)
2. 40% w/v Ammonium sulphate (AnalaR)
3. Sodium chloride (AnalaR)
4. Concentrated hydrochloric acid (AnalaR) Specific gravity 1.18
5. 1% w/v diphenylamine (AnalaR) in concentrated sulphuric acid (Specific gravity 1.84)
6. 2% w/v potassium ferricyanide
7. 0.075 M zinc sulphate (AnalaR)
8. Wash solution (distilled water (840 cm^3), sodium chloride (AnalaR) (240 g), concentrated hydrochloric acid (80 cm^3)).

Method

a) Preparation of Calibration Curves

The ethylene oxide-propylene oxide copolymer was accurately weighed into a conical flask. Distilled water (100 cm^3), hydrochloric acid (10 cm^3) and sodium chloride (15 g) were added and the mixture shaken mechanically for 30 minutes. The mixture was allowed to stand until any foam produced had dispersed. Potassium ferrocyanide (5 cm^3) was added and the flask shaken automatically for 10 minutes. After standing for 5 minutes the precipitate was filtered (Whatman 541 filter paper). Wash solution (Reagent 8) (25 cm^3) was added to the flask and swirled. This was washed over the precipitate.

After washing, ammonium sulphate solution (Reagent 2) (5 cm^3), potassium ferricyanide (Reagent 6) (6 drops) and diphenylamine (Reagent 5) (6 drops) were added to the filtrate and the mixture immediately titrated against 0.075M zinc sulphate. The solution becomes pale green and at the end point it changed to blue-violet. The flask was vigorously swirled during titration.

b) Determination of Polymer Concentration in the Fermentation Medium

As described in Section 6.3 yeast was separated from the medium by centrifugation and decantation. After decanting the fermentation medium (100 cm^3) was used in place of distilled water in the above method.

In the case of the more hydrophobic polymers when used at only 5 mg in the fermentation medium a larger aliquot (200 cm^3) was used for the purpose of sensitivity.

Appendix 1

General data on Saccaromyces Cerevisiae NCYC 990 is given below abstracted from data sheet record number 396 of the National Collection of Yeast Cultures dated 29 October, 1984.

General

Name:	Saccharomyces Cerevisiae
Number:	990
Collection:	NCYC
Habitat:	Wine/beer/fermentation industries

Cells

Shape:	Oval
Breadth in broth:	3 - 6 μ
Length in broth:	7 - 20 μ
Arrangement:	Pairs
Division:	Multipolar budding
Sexual Spores:	Smooth ascospores

Fermentation

This yeast is known to ferment the following carbohydrates:

Glucose
Galactose
Sucrose
Maltose
Raffinose
Melezitose

Miscellaneous

Maximum growth temperature:	35.5°C
Minimum growth temperature:	12.5°C
Ammonium sulphate assimilation:	present
Inositol assimilation:	absent
Ethanol assimilation:	present

Appendix 2

Table of surface tensions and interfacial surface tensions of ethylene oxide-propylene oxide copolymers at a concentration of 0.001% weight/volume in fermentation medium.

SAMPLE	SURFACE TENSION / dyne cm^{-1}	INTERFACIAL SURFACE TENSION / dyne cm^{-1}
Blank	72.4	-
Pluronic L31P	56.0	26.5
Pluronic L61P	47.3	19.6
Pluronic L62P	48.3	21.8
Pluronic L63P	49.0	19.8
Pluronic L64P	55.9	21.9
Pluronic L81P	49.0	14.8
Pluronic L92P	47.0	14.8
Pluronic L101P	41.2	11.7
Pluronic L121P	35.6	9.8

1. Surface tension and interfacial surface tension made with a Du Nuoy tensionmeter at 20°C.

2. Interfacial surface tensions recorded between culture medium and Nujol (liquid parafin).

3. Surface tension of water at 20°C is 73.5 dyne cm^{-1}.

Key to Appendices 3 - 12, 22 - 31 and 42 - 51

Key	Polymer weight / mg
x ——— x	5
+ ——— +	15
△ ——— △	50
⊙ ——— ⊙	75
● ——— ●	100

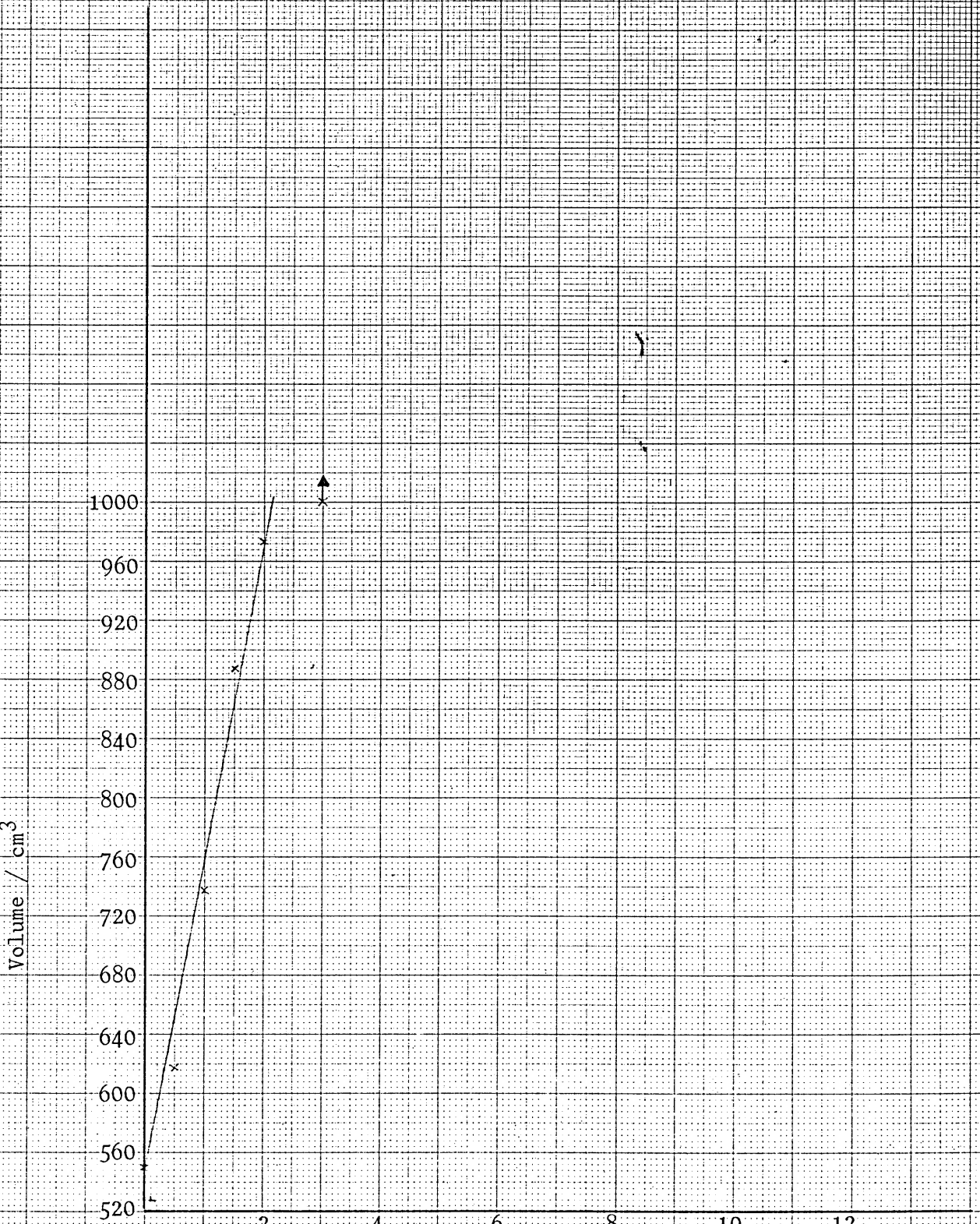
N.B. i) All of the results in the following appendices are the mean of three values.

ii) All of the standard deviations in the following appendices are derived using weighted values from three fermentations for each perameter (e.g. the standard deviations in appendix 71 were obtained using 15 weighted values for yeast yield, i.e. 3 values for each polymer weight used).

Appendix 3

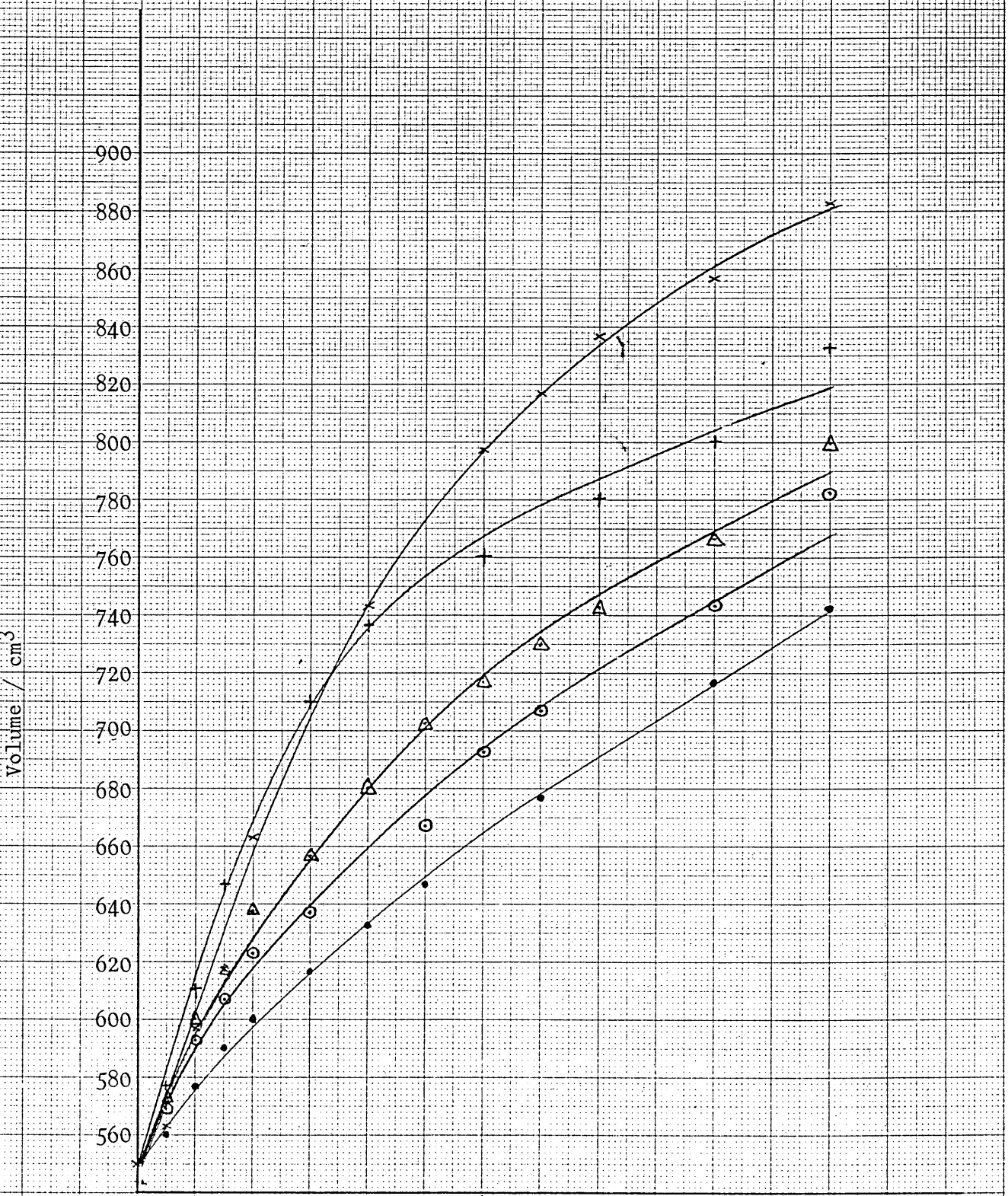
No Polymer

Graph of fermenter contents volume against time



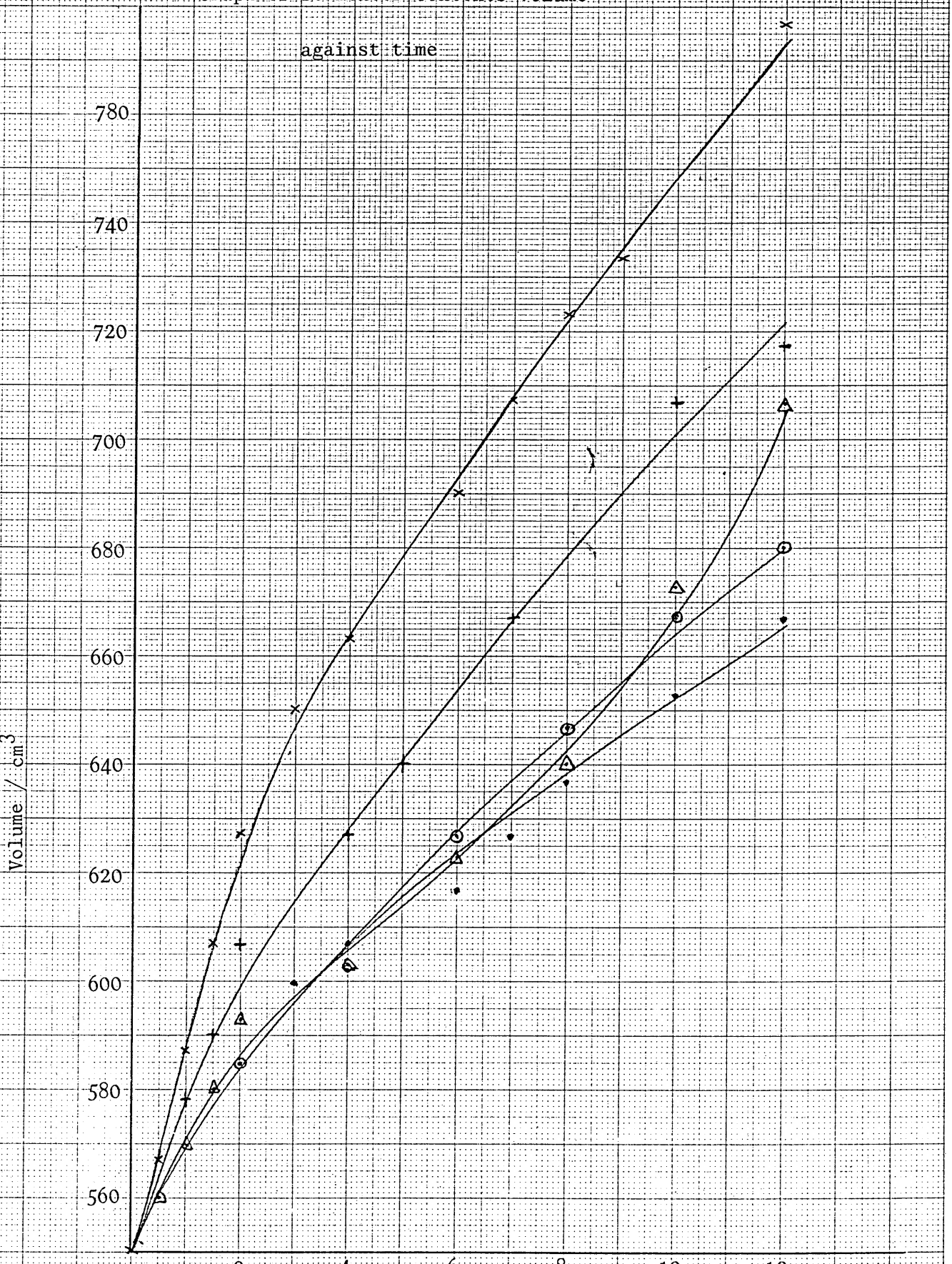
Pluronic L31P

Graph of fermenter contents volume against time



Pluronic L61P

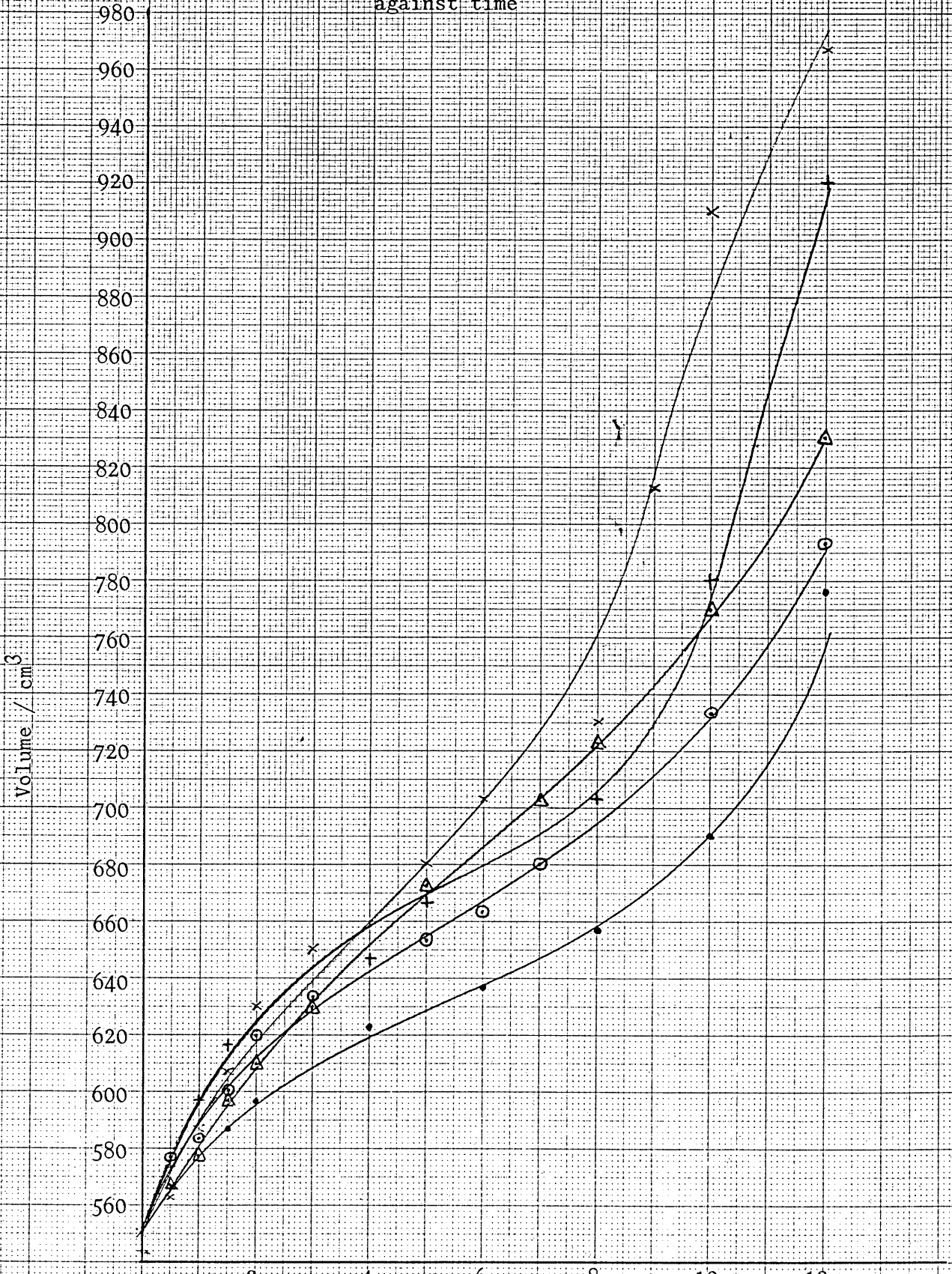
Graph of fermenter contents volume against time



Appendix 6

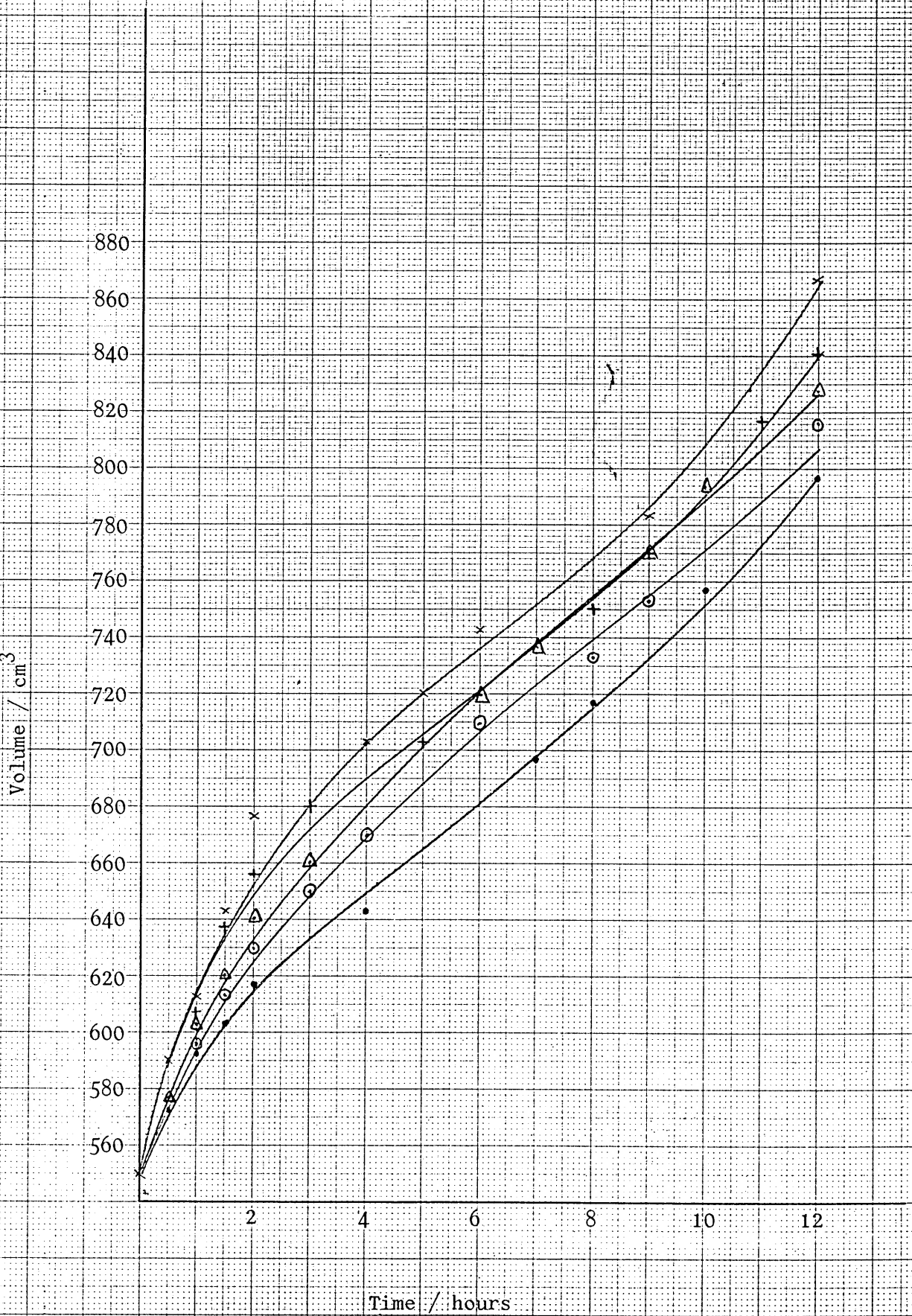
Pluronic L62P

Graph of fermenter contents volume against time



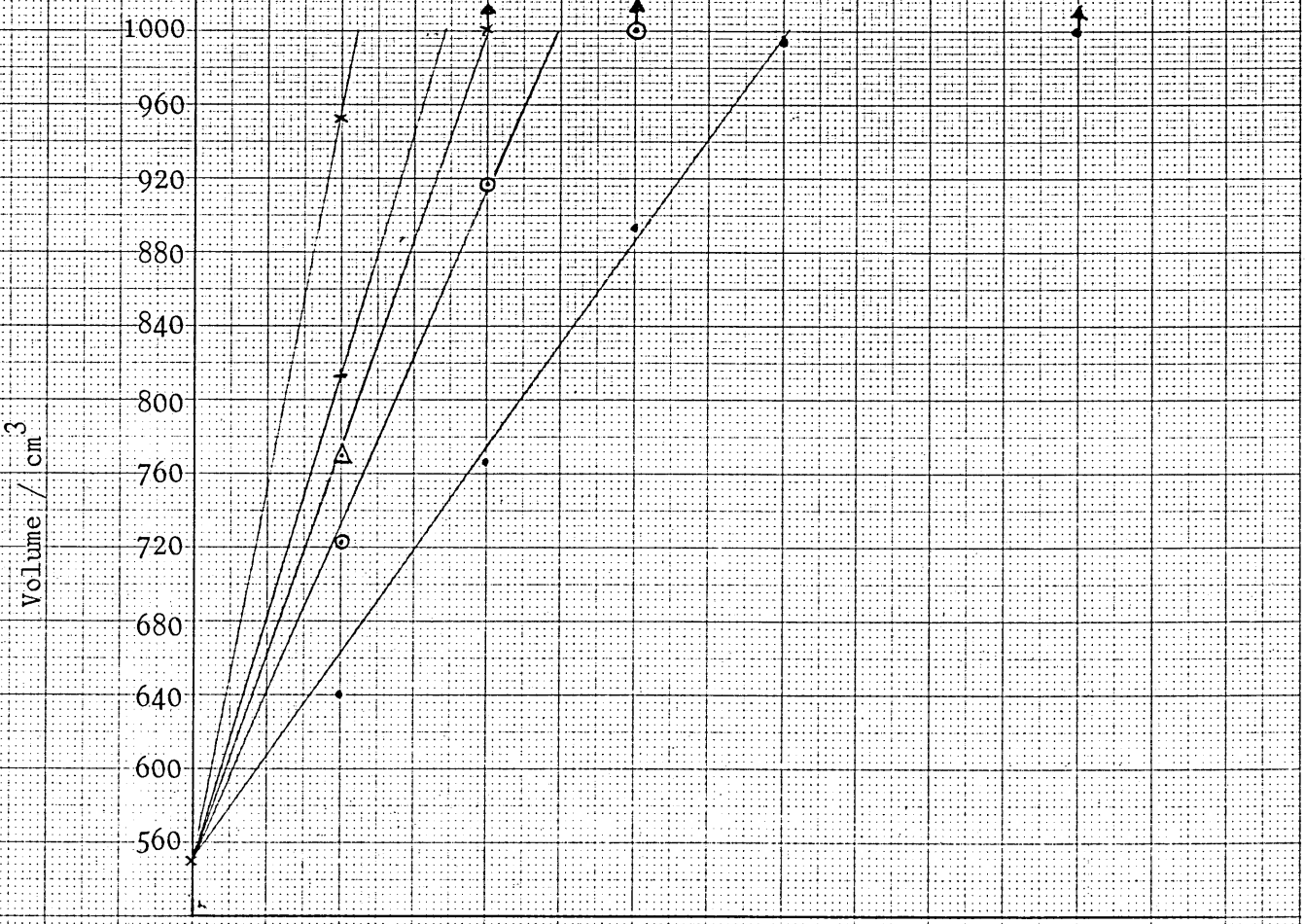
Pluronic L63P

Graph of fermenter contents volume against time



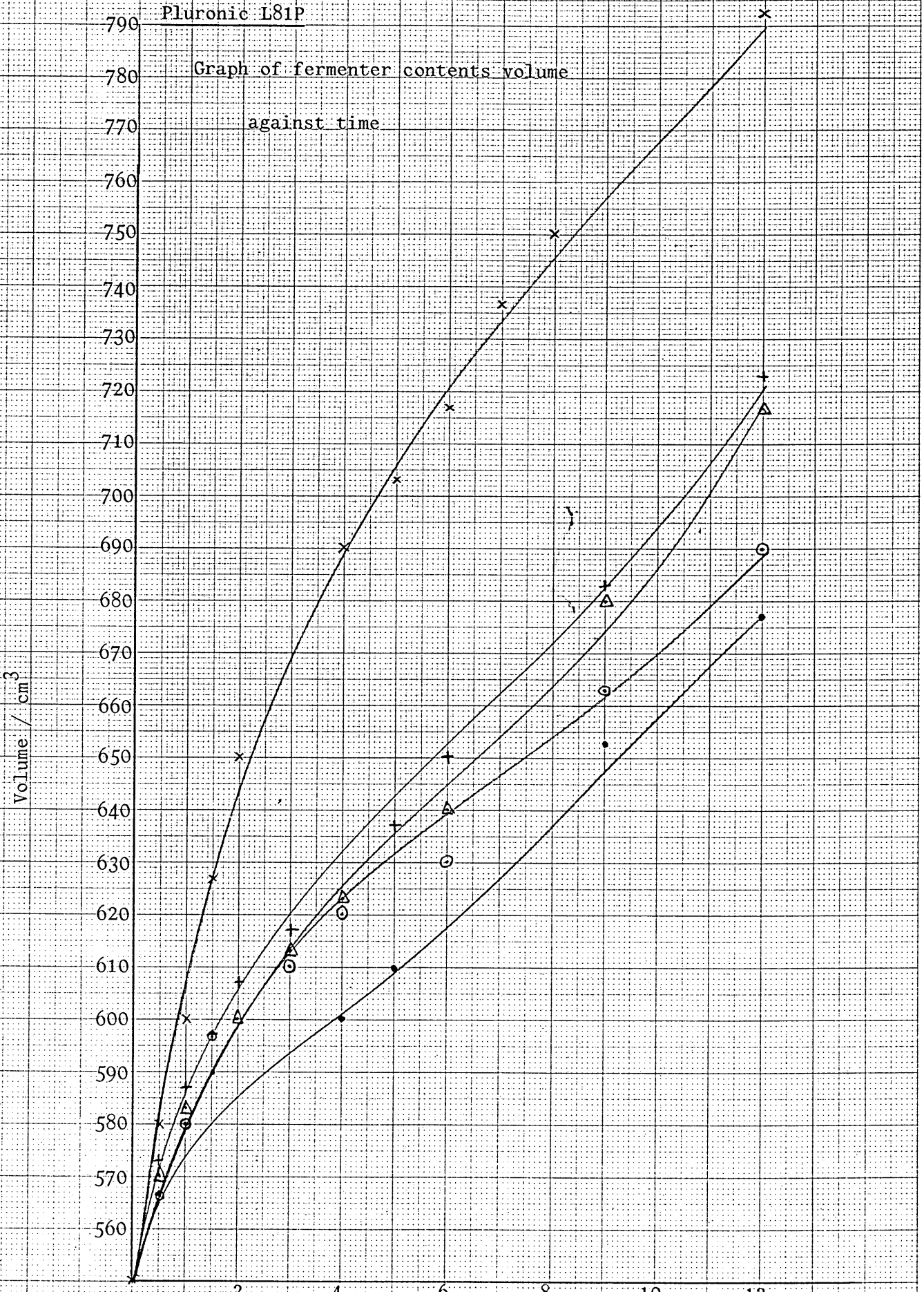
Pluronic L64P

Graph of fermenter contents volume against time



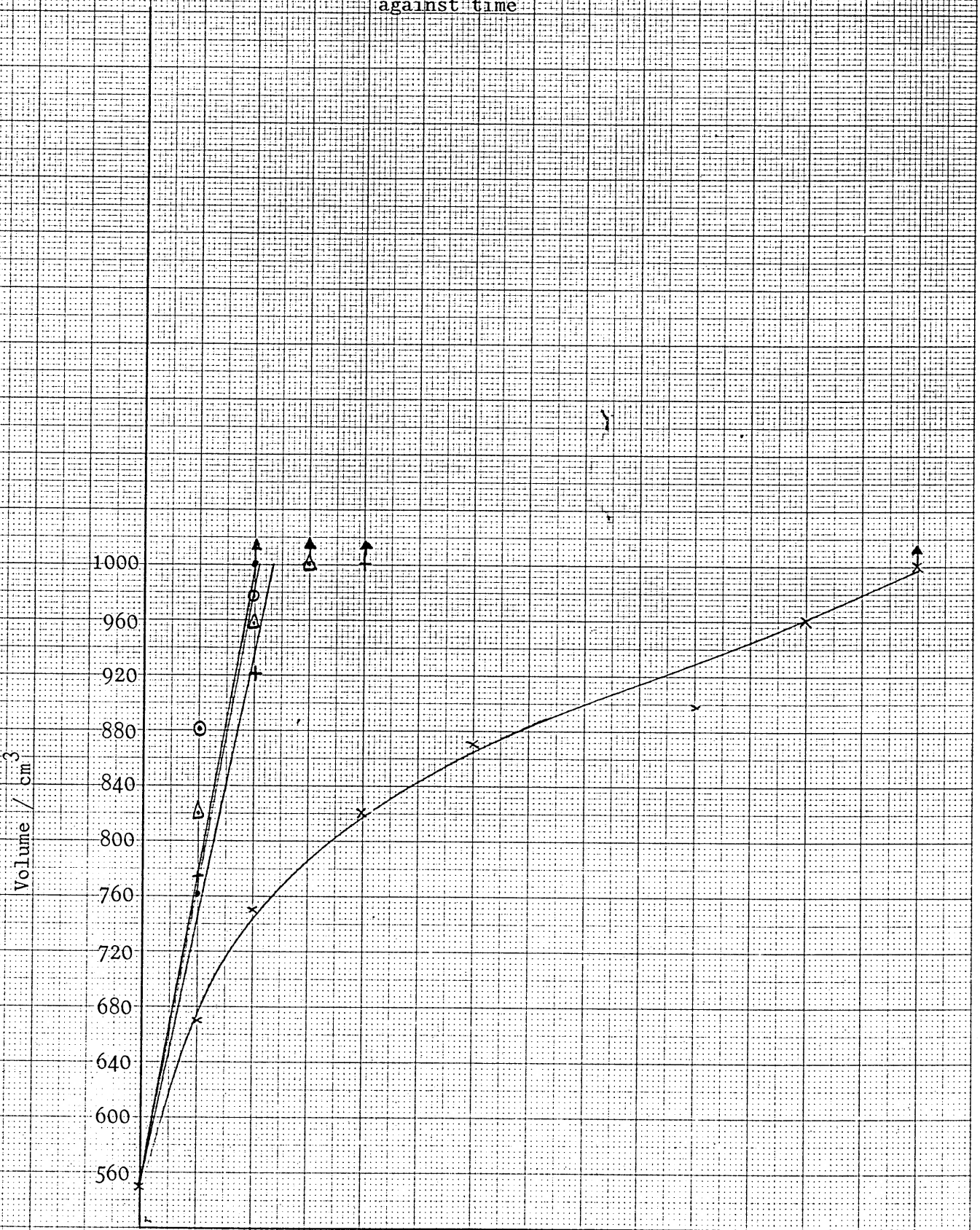
Pluronic L81P

Graph of fermenter contents volume against time



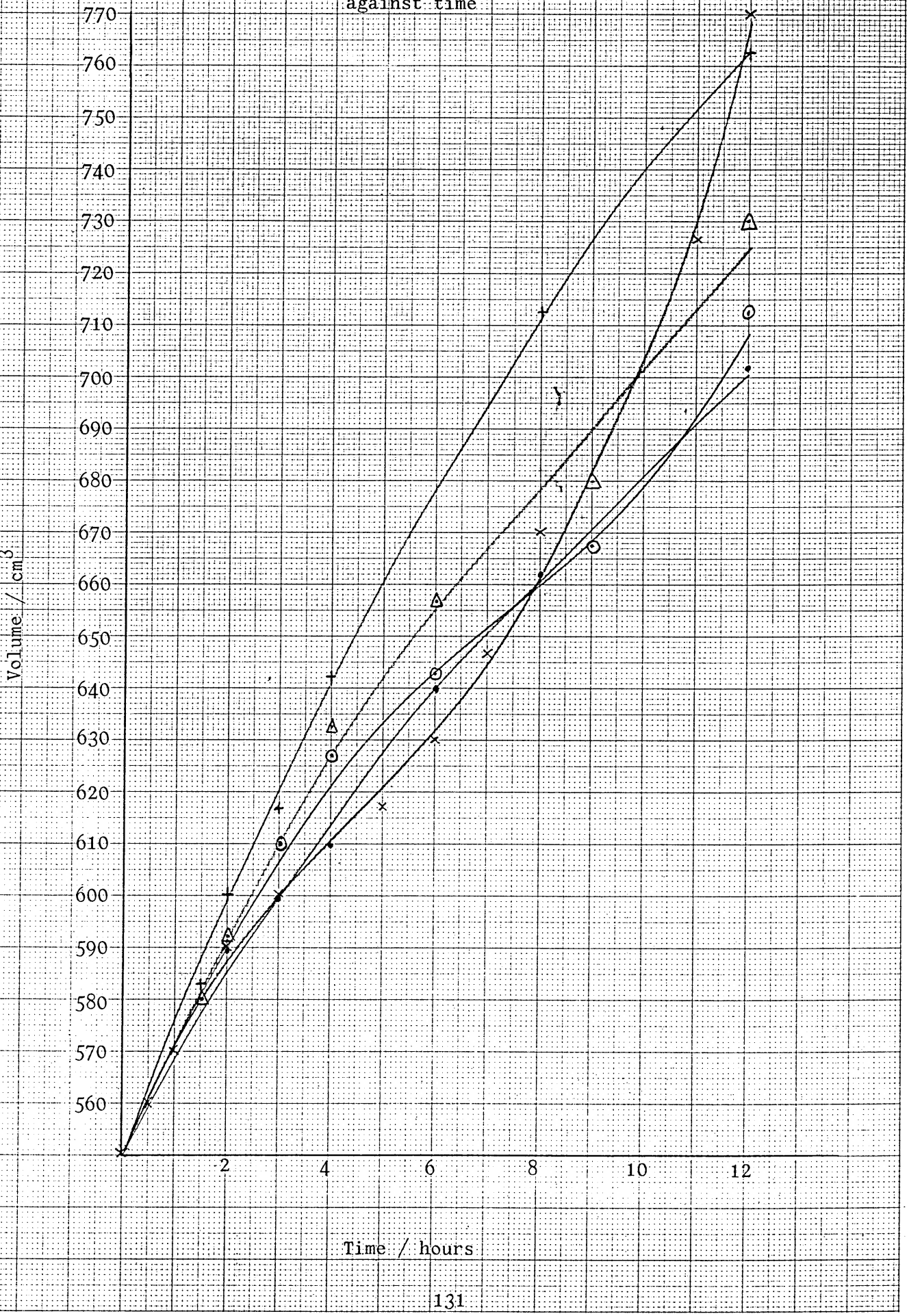
Pluronic L92P

Graph of fermenter contents volume against time



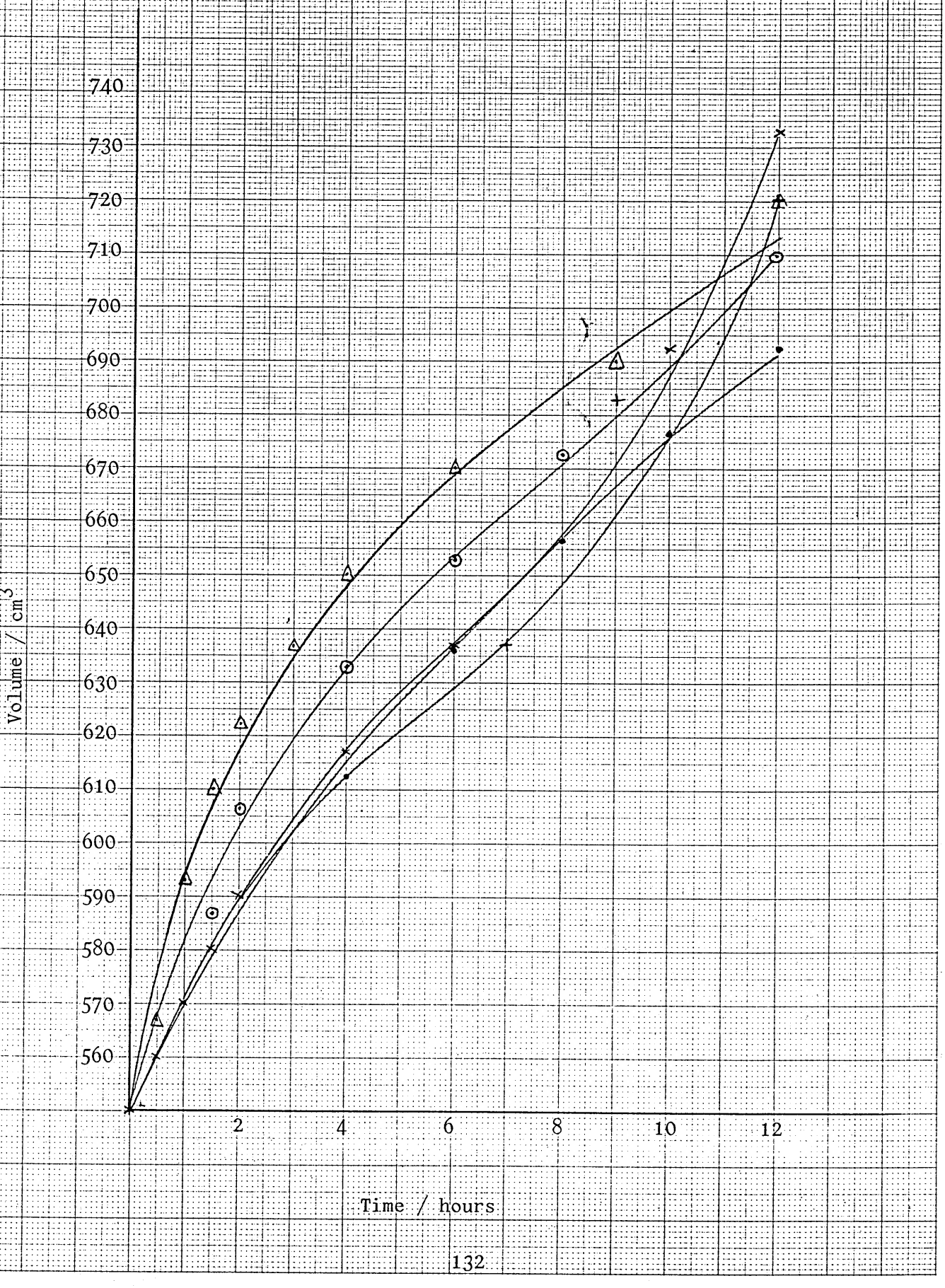
Pluronic L101P

Graph of fermenter contents volume against time



Pluronic L121P

Graph of fermenter contents volume against time



Appendix 13

Variation of fermenter contents volume with time.

Pluronic L31P

TIME/HOURS	FERMENTER CONTENTS VOLUME / cm^3 USING THE FOLLOWING POLYMER WEIGHT / mg					
	NO POLYMER PRESENT	5	15	50	75	100
0.0	550	550	550	550	550	550
0.5	607	563	577	573	570	560
1.0	737	597	610	600	593	577
1.5	893	617	647	617	607	590
2.0	973	663	663	637	623	600
3.0	>1000	-	710	657	637	617
4.0	>1000	743	737	680	-	633
5.0	>1000	-	-	703	667	647
6.0	>1000	797	760	717	693	-
7.0	>1000	817	-	730	707	677
8.0	>1000	837	780	743	-	-
9.0	>1000	-	-	-	-	-
10.0	>1000	857	800	767	743	717
11.0	>1000	-	-	-	-	-
12.0	>1000	883	833	800	783	743

All results in appendices 3 - 78 are the mean of three values

Sample standard deviation σ_{n-1} = 7.84×10^{-3} cm^3 No polymer present

Population Standard deviation σ_n = 7.82×10^{-3} cm^3 No polymer present

σ_{n-1} = 0.0123 cm^3 Pluronic L31P

σ_n = 0.0119 cm^3 Pluronic L31P

Appendix 14

Variation of fermenter contents volume with time

Pluronic L61P

TIME/HOURS	FERMENTER CONTENTS VOLUME/cm^3 USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	550	550	550	550	550
0.5	567	567	560	560	560
1.0	587	577	570	570	570
1.5	607	590	580	580	580
2.0	627	607	593	590	590
3.0	650	-	-	-	600
4.0	663	627	603	603	607
5.0	-	640	-	-	-
6.0	690	-	623	627	617
7.0	707	667	-	-	627
8.0	723	-	640	647	637
9.0	733	-	-	-	-
10.0	-	707	673	667	653
11.0	-	-	-	-	-
12.0	777	717	707	680	667

$\sigma_n - 1 = 6.15 \times 10^{-3}\ cm^3$

$\sigma_n = 6.13 \times 10^{-3}\ cm^3$

Appendix 15

Variation of fermenter contents volume with time.

Pluronic L62P

TIME/HOURS	FERMENTER CONTENTS VOLUME/cm^3 USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	550	550	550	550	550
0.5	563	573	567	577	563
1.0	587	597	577	583	577
1.5	607	617	597	600	587
2.0	630	630	610	620	597
3.0	650	-	630	633	-
4.0	-	647	-	-	623
5.0	680	667	673	653	-
6.0	703	-	-	663	637
7.0	-	-	703	680	-
8.0	730	703	723	-	657
9.0	813	-	-	-	-
10.0	910	780	770	733	690
11.0	-	-	-	-	-
12.0	967	920	830	793	777

$$\sigma_{n-1} = 9.47 \times 10^{-3}\ cm^3$$

$$\sigma_n = 9.44 \times 10^{-3}\ cm^3$$

Appendix 16

Variation of fermenter contents volume with time.

Pluronic L63P

TIME/HOURS	FERMENTER CONTENTS VOLUME/cm^3 USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	550	550	550	550	550
0.5	590	590	577	577	573
1.0	613	607	603	597	593
1.5	643	637	620	613	603
2.0	677	657	640	630	617
3.0	-	680	660	650	-
4.0	703	-	-	670	643
5.0	720	703	-	-	-
6.0	743	-	720	710	-
7.0	-	-	737	-	697
8.0	-	750	-	733	717
9.0	783	770	770	753	-
10.0	-	-	793	-	757
11.0	-	817	-	-	-
12.0	867	840	827	817	797

$$\sigma_n - 1 = 7.71 \times 10^{-3}\ cm^3$$

$$\sigma_n = 7.68 \times 10^{-3}\ cm^3$$

Appendix 17

Variation of fermenter contents volume with time.

Pluronic L64P

TIME/HOURS	FERMENTER CONTENTS VOLUME/cm^3 USING THE FOLLOWING POLYMER WEIGHTS / mg				
	5	15	50	75	100
0.0	550	550	550	550	550
0.5	953	813	770	723	640
1.0	>1000	>1000	>1000	917	767
1.5	>1000	>1000	>1000	>1000	893
2.0	>1000	>1000	>1000	>1000	993
3.0	>1000	>1000	>1000	>1000	>1000
4.0	>1000	>1000	>1000	>1000	>1000
5.0	>1000	>1000	>1000	>1000	>1000
6.0	>1000	>1000	>1000	>1000	>1000
7.0	>1000	>1000	>1000	>1000	>1000
8.0	>1000	>1000	>1000	>1000	>1000
9.0	>1000	>1000	>1000	>1000	>1000
10.0	>1000	>1000	>1000	>1000	>1000
11.0	>1000	>1000	>1000	>1000	>1000
12.0	>1000	>1000	>1000	>1000	>1000

$$\sigma_n - 1 = 14.00 \times 10^{-3}\ cm^3$$

$$\sigma_n = 13.86 \times 10^{-3}\ cm^3$$

Appendix 18

Variation of fermenter contents volume with time.

Pluronic L81P

TIME/HOURS	FERMENTER CONTENTS VOLUME/cm^3 USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	550	550	550	550	550
0.5	580	573	570	567	567
1.0	600	587	583	580	580
1.5	627	597	-	597	590
2.0	650	607	600	-	-
3.0	-	617	613	610	-
4.0	690	-	623	620	600
5.0	703	637	-	-	610
6.0	717	650	640	630	-
7.0	737	-	-	-	-
8.0	750	-	-	-	-
9.0	-	683	680	663	-
10.0	-	-	-	-	653
11.0	-	-	-	-	-
12.0	793	723	717	690	177

$\sigma_{n-1} = 10.30 \times 10^{-3}\ cm^3$

$\sigma_n = 10.30 \times 10^{-3}\ cm^3$

Appendix 19

Variation of fermenter contents volume with time.

Pluronic L92P

TIME/HOURS	FERMENTER CONTENTS VOLUME/cm^3 USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	550	550	550	550	550
0.5	670	773	820	880	760
1.0	750	920	957	977	> 1000
1.5	–	–	> 1000	> 1000	> 1000
2.0	820	> 1000	> 1000	> 1000	> 1000
3.0	870	> 1000	> 1000	> 1000	> 1000
4.0	–	> 1000	> 1000	> 1000	> 1000
5.0	897	> 1000	> 1000	> 1000	> 1000
6.0	960	> 1000	> 1000	> 1000	> 1000
7.0	> 1000	> 1000	> 1000	> 1000	> 1000
8.0	> 1000	> 1000	> 1000	> 1000	> 1000
9.0	> 1000	> 1000	> 1000	> 1000	> 1000
10.0	> 1000	> 1000	> 1000	> 1000	> 1000
11.0	> 1000	> 1000	> 1000	> 1000	> 1000
12.0	> 1000	> 1000	> 1000	> 1000	> 1000

$$\sigma_n - 1 = 11.90 \times 10^{-3}\ cm^3$$

$$\sigma_n = 11.80 \times 10^{-3}\ cm^3$$

Appendix 20

Variation of fermenter contents volume with time.

Pluronic L101P

TIME/HOURS	FERMENTER CONTENTS VOLUME/cm^3 USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	550	550	550	550	550
0.5	560	560	560	560	560
1.0	570	570	-	570	570
1.5	-	583	580	580	580
2.0	590	600	593	590	590
3.0	600	617	-	610	600
4.0	-	643	633	627	610
5.0	617	-	-	-	-
6.0	630	-	657	643	640
7.0	647	-	-	-	-
8.0	670	713	-	-	663
9.0	-	-	680	667	-
10.0	-	-	-	-	-
11.0	727	-	-	-	-
12.0	770	763	730	713	703

$$\sigma_{n-1} = 6.17 \times 10^{-3} \text{ cm}^3$$

$$\sigma_n = 6.15 \times 10^{-3} \text{ cm}^3$$

Appendix 21

Variation of fermenter contents volume with time.

Pluronic L121P

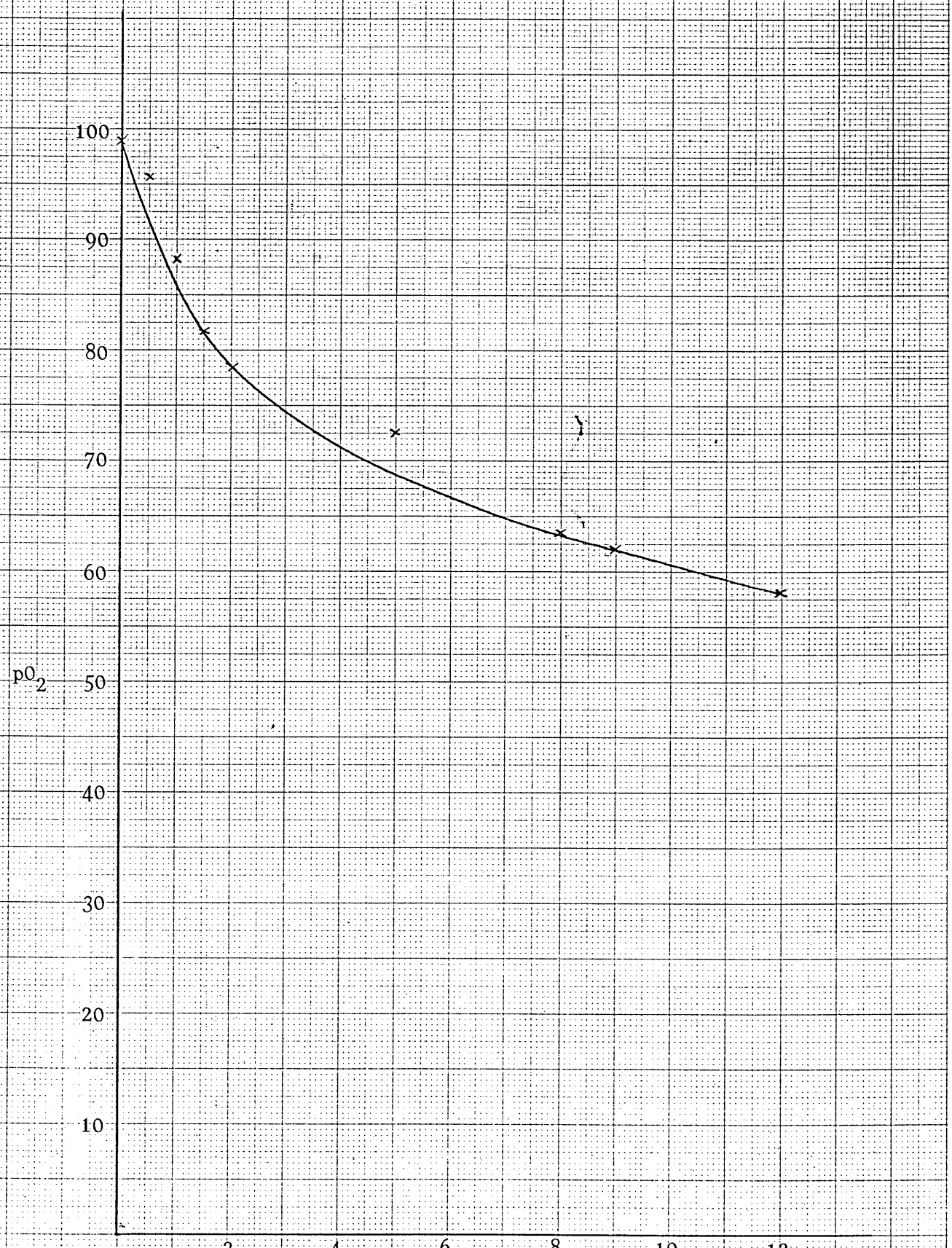
TIME/HOURS	FERMENTER CONTENTS VOLUME/cm^3 USING THE FOLLOWING POLYMER WEIGHTS / mg				
	5	15	50	75	100
0.0	550	550	550	550	550
0.5	560	560	567	560	560
1.0	570	570	593	570	570
1.5	580	580	610	587	580
2.0	590	590	623	607	590
3.0	-	-	637	-	-
4.0	617	-	650	633	613
5.0	-	-	-	-	-
6.0	637	-	670	653	637
7.0	-	637	-	-	-
8.0	-	-	-	673	657
9.0	-	683	690	-	-
10.0	693	-	-	-	677
11.0	-	-	-	-	-
12.0	733	720	720	710	693

$$\sigma_n - 1 = 7.30 \times 10^{-3} \text{ cm}^3$$

$$\sigma_n = 7.27 \times 10^{-3} \text{ cm}^3$$

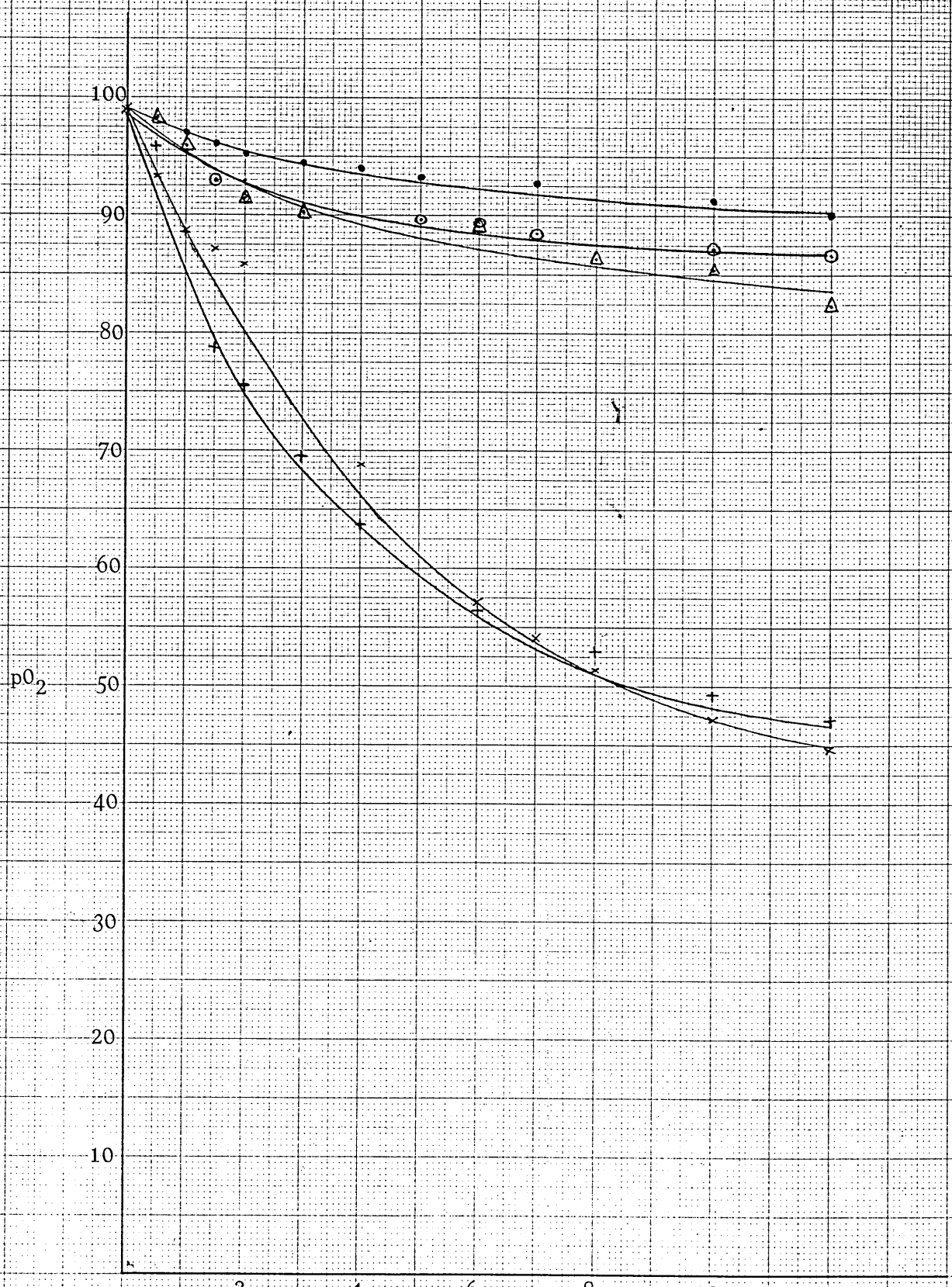
No Polymer Present

Graph of pO_2 against time



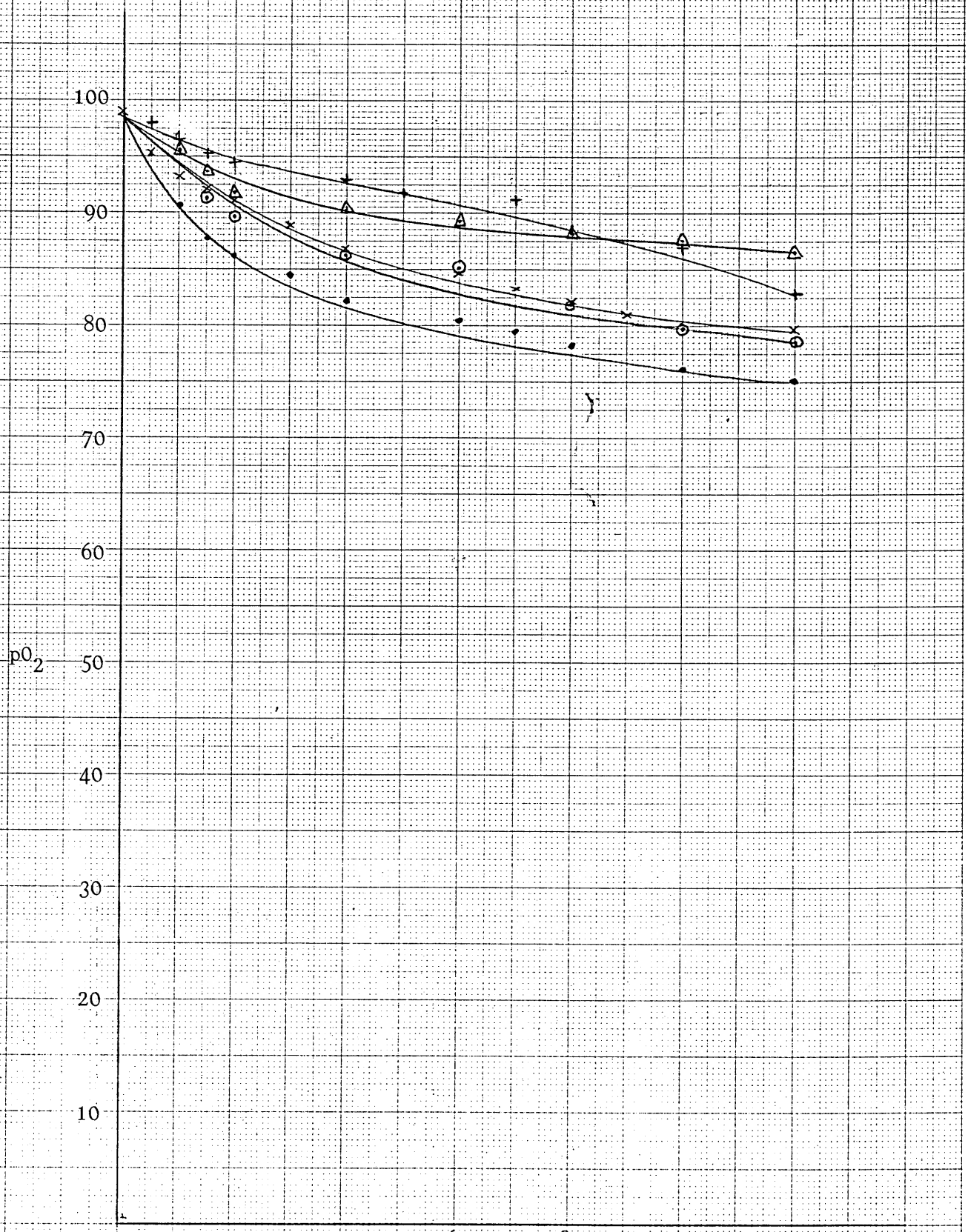
Pluronic L31P

Graph of pO_2 against time



Pluronic L61P

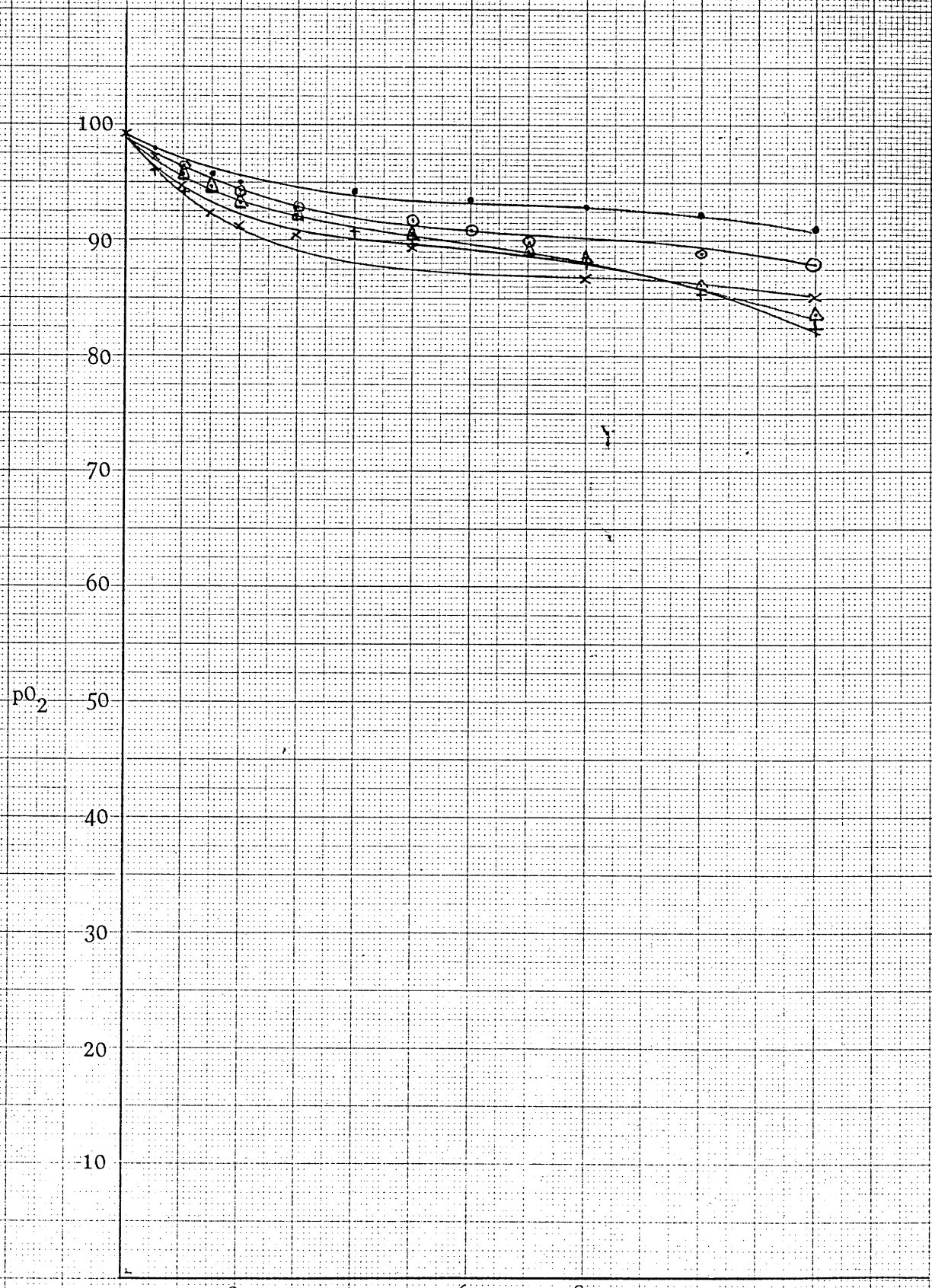
Graph of pO_2 against time



Appendix 23

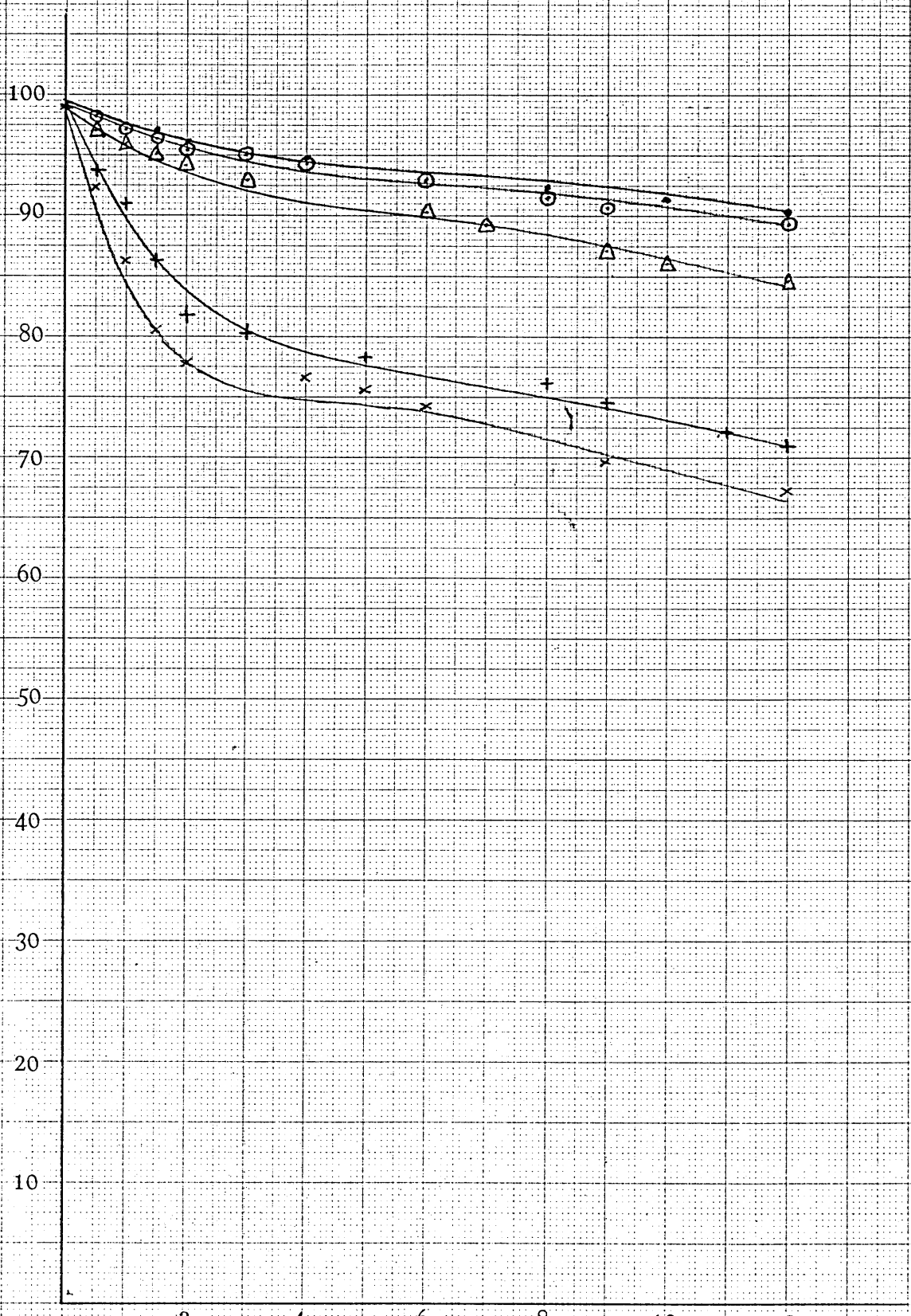
Pluronic L62P

Graph of pO_2 against time



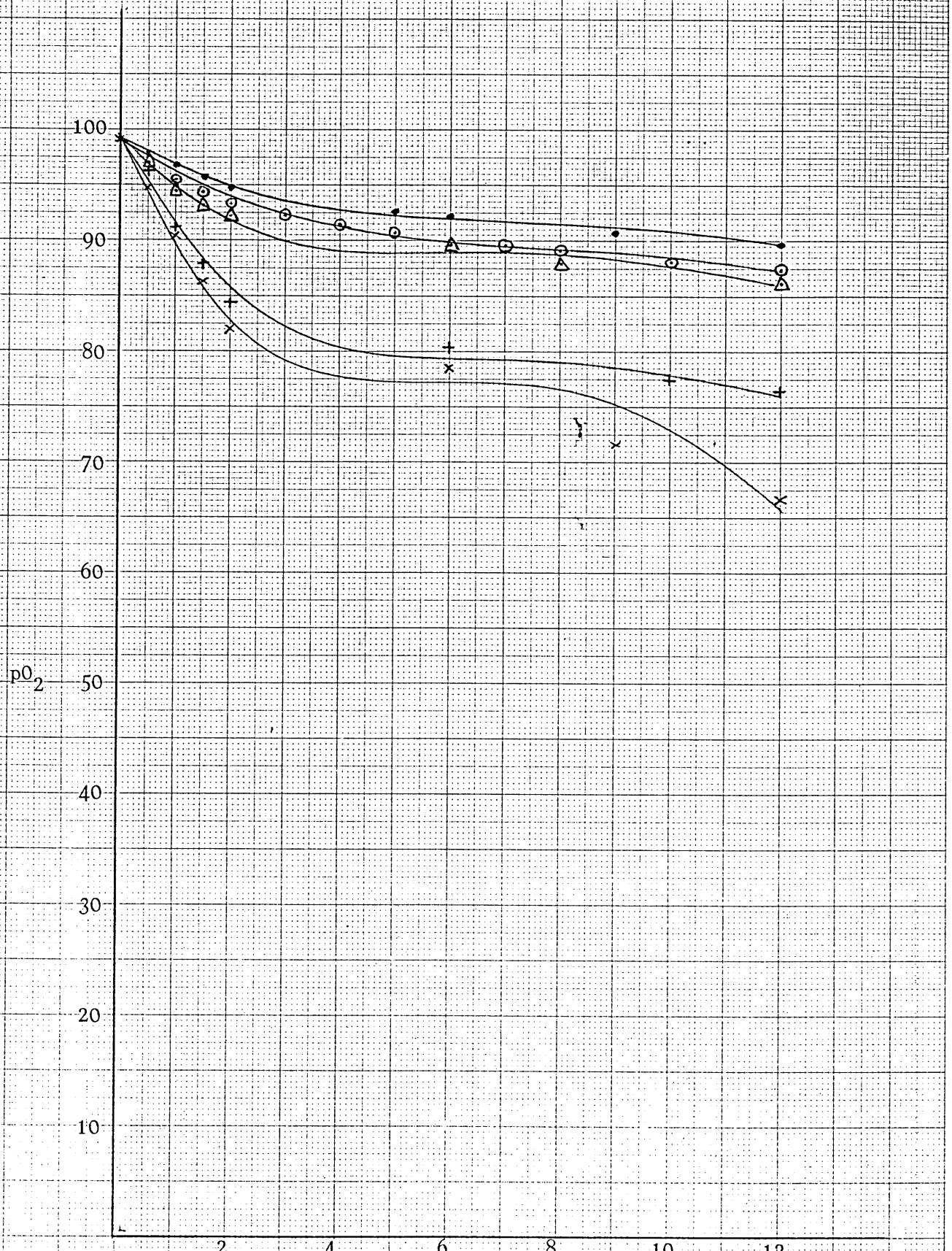
Pluronic L63P

Graph of pO_2 against time



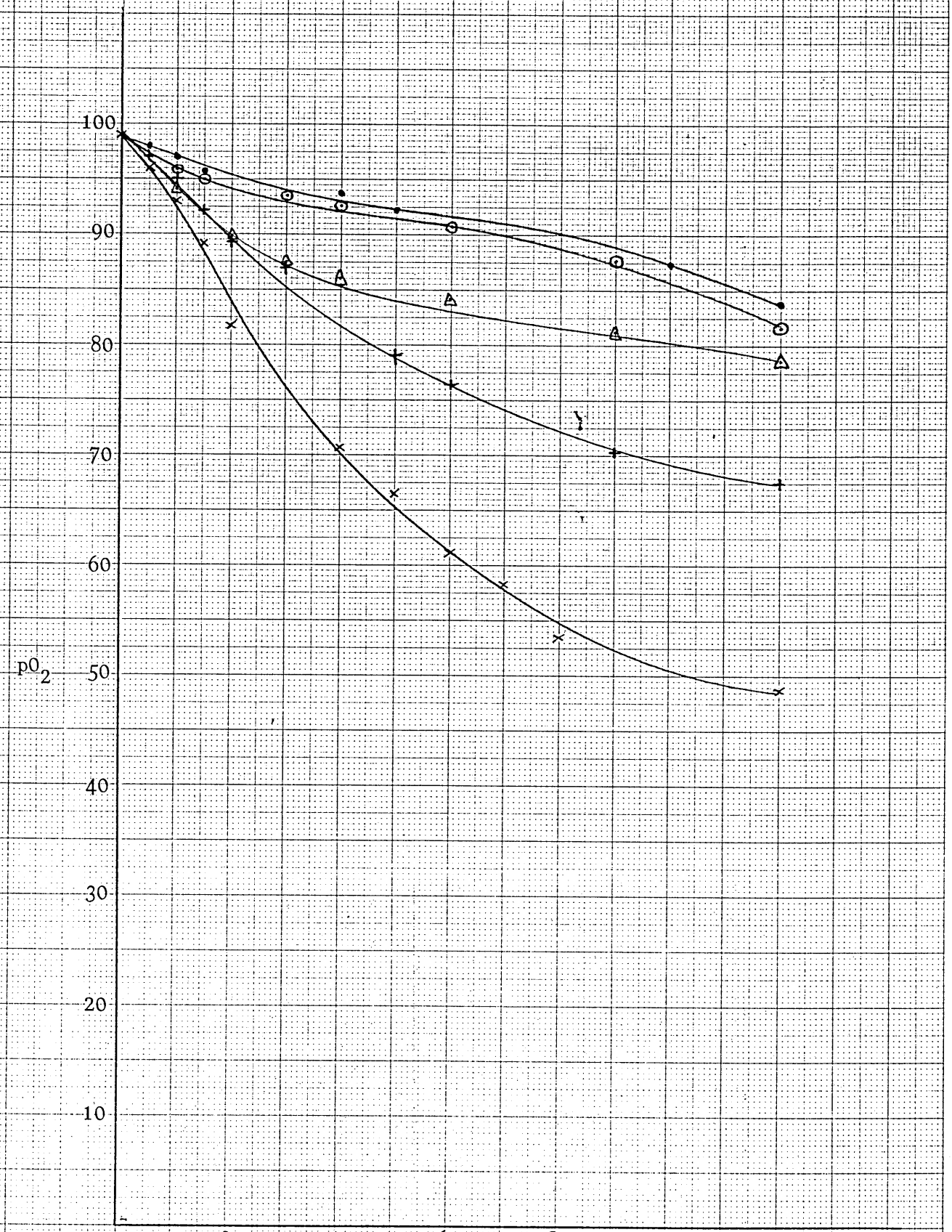
Pluronic L64P

Graph of pO_2 against time



Pluronic L81P

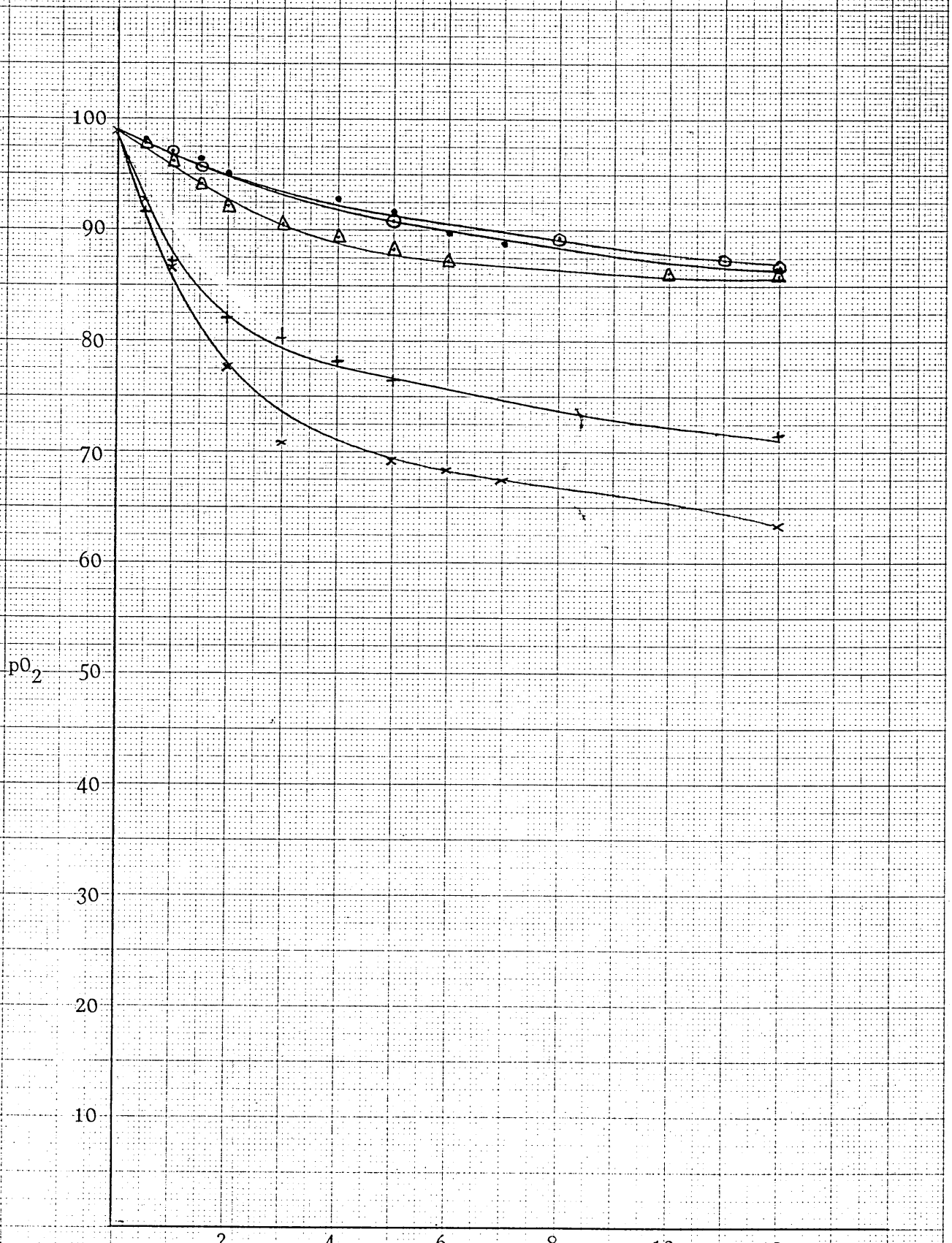
Graph of pO_2 against time



Appendix B7

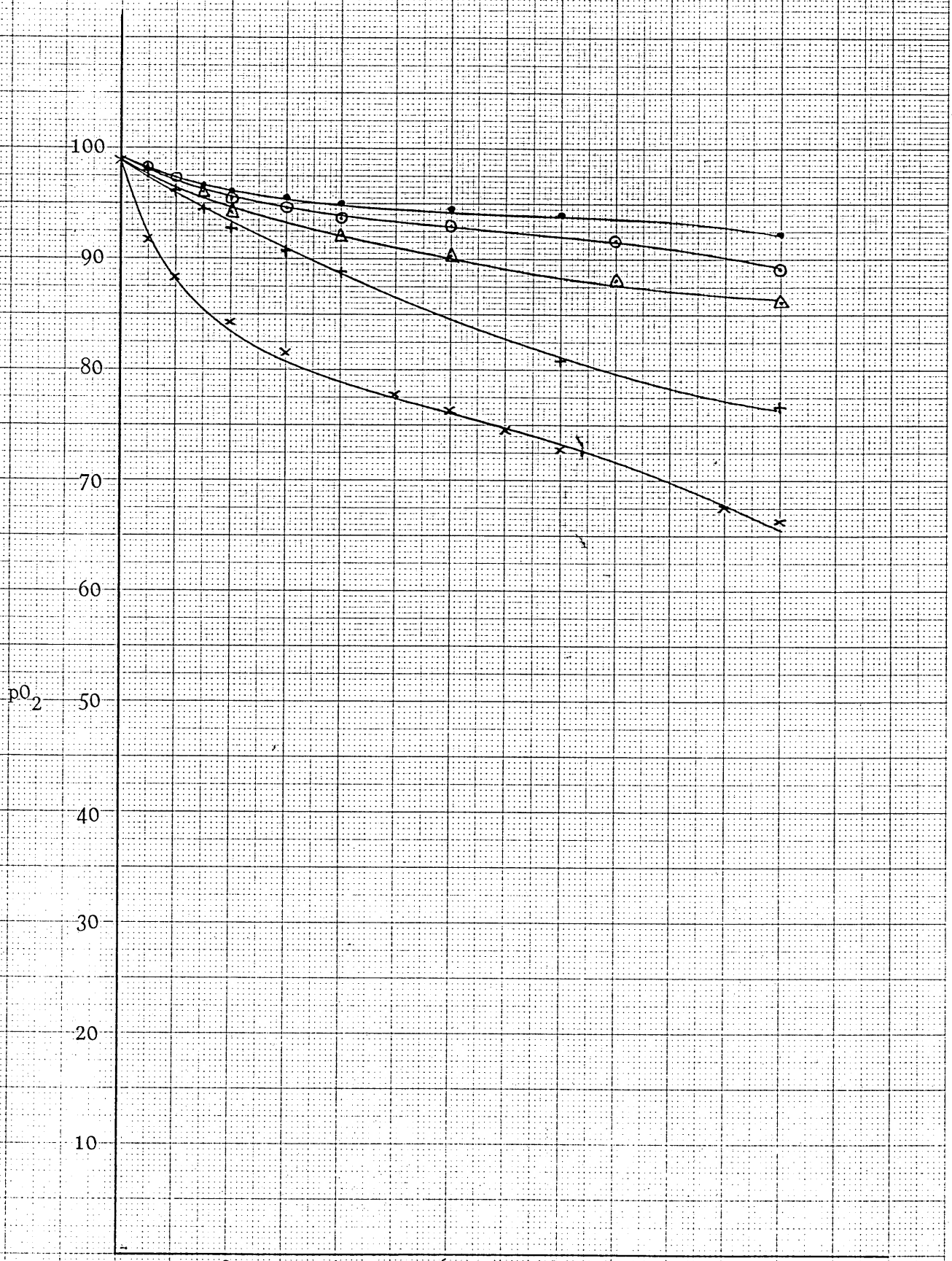
Pluronic L92P

Graph of pO_2 against time



Pluronic L101P

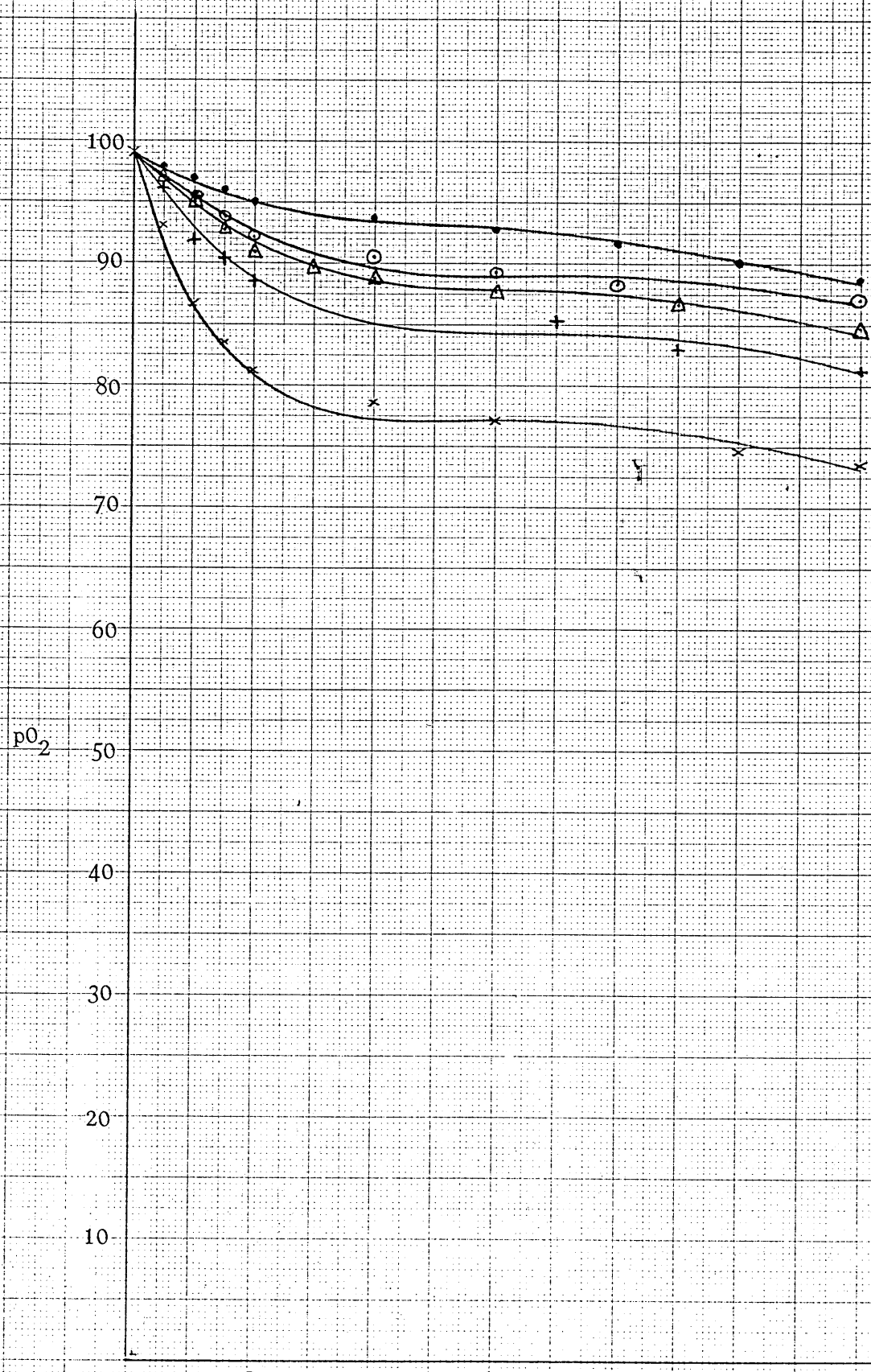
Graph of pO_2 against time



Appendix 31

Pluronic L121P

Graph of pO_2 against time



Appendix 32

Variation of dissolved oxygen concentration with time.

Pluronic L31P

TIME/HOURS	DISSOLVED OXYGEN CONCENTRATION/% IN THE FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	99.0	99.2	99.0	99.0	99.0
0.5	93.2	95.7	98.3	97.8	98.0
1.0	88.8	88.5	95.8	95.7	97.0
1.5	87.0	78.7	-	92.7	96.0
2.0	85.8	75.5	91.3	91.8	95.2
3.0	-	69.5	90.3	90.5	94.5
4.0	68.7	63.8	-	-	94.0
5.0	-	-	-	89.7	93.7
6.0	57.0	56.5	89.0	89.0	-
7.0	54.0	-	-	88.3	92.8
8.0	51.3	53.0	86.3	-	-
9.0	-	-	-	-	-
10.0	47.3	49.3	85.2	87.0	91.3
11.0	-	-	-	-	-
12.0	44.8	47.3	82.3	86.5	90.0

$$\sigma_{n-1} = 7.55 \times 10^{-3} \%$$

$$\sigma_{n} = 7.52 \times 10^{-3} \%$$

Appendix 33

Variation of dissolved oxygen concentration with time.

Pluronic L61P

TIME/HOURS	DISSOLVED OXYGEN CONCENTRATION/% IN THE FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	99.0	99.2	99.0	99.2	99.0
0.5	95.2	98.0	97.0	97.2	95.3
1.0	93.3	96.5	95.5	95.5	90.5
1.5	92.0	95.2	93.7	91.2	87.8
2.0	91.0	94.3	91.7	89.8	86.2
3.0	88.8	-	-	-	84.5
4.0	86.8	93.2	90.3	87.2	82.3
5.0	-	92.3	-	-	-
6.0	84.5	-	89.2	85.0	80.5
7.0	83.2	91.7	-	-	79.5
8.0	82.0	-	88.3	82.2	78.3
9.0	81.0	-	-	-	-
10.0	-	86.8	87.5	79.8	76.2
11.0	-	-	-	-	-
12.0	79.8	82.8	86.5	78.5	75.0

$\sigma_n - 1 = 5.26 \times 10^{-3}$ %

$\sigma_n = 5.24 \times 10^{-3}$ %

Appendix 34

Variation of dissolved oxygen concentration with time.

Pluronic L62P

TIME/HOURS	DISSOLVED OXYGEN CONCENTRATION/% IN THE FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	99.3	99.0	98.7	99.0	99.0
0.5	97.2	96.0	97.2	97.2	97.8
1.0	94.8	94.2	95.8	96.5	96.7
1.5	92.3	92.3	94.5	94.8	95.8
2.0	91.2	91.3	93.2	93.8	94.8
3.0	90.3	-	92.2	92.8	-
4.0	-	90.7	-	-	94.2
5.0	89.3	89.5	90.5	91.7	-
6.0	-	-	-	90.8	93.5
7.0	-	-	88.8	89.8	-
8.0	86.7	88.0	87.8	-	93.2
9.0	-	-	-	-	-
10.0	-	85.3	86.0	88.8	92.3
11.0	-	-	-	-	-
12.0	85.0	82.3	83.8	88.0	91.0

$\sigma_{n-1} = 5.87 \times 10^{-3}$ %

$\sigma_{n} = 5.85 \times 10^{-3}$ %

Appendix 35

Variation of dissolved oxygen concentration with time.

Pluronic L63P

TIME/HOURS	DISSOLVED OXYGEN CONCENTRATION/% IN THE FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	99.0	99.2	99.2	99.2	99.0
0.5	92.3	93.8	97.2	98.2	98.2
1.0	86.3	91.0	96.2	97.0	97.3
1.5	80.5	86.3	95.3	96.3	96.5
2.0	77.7	81.7	94.3	95.3	95.7
3.0	-	80.3	93.2	95.0	-
4.0	76.5	-	-	94.2	94.5
5.0	75.5	78.3	-	-	-
6.0	74.2	-	90.3	93.0	-
7.0	-	-	89.3	-	93.2
8.0	-	76.3	-	91.5	92.3
9.0	69.7	74.5	87.3	90.7	-
10.0	-	-	86.2	-	91.2
11.0	-	72.3	-	-	-
12.0	67.3	71.0	84.8	89.3	90.2

$\sigma_{n-1} = 5.00 \times 10^{-3}$ %

$\sigma_{n} = 4.99 \times 10^{-3}$ %

Appendix 36

Variation of dissolved oxygen concentration with time.

Pluronic L64P

TIME/HOURS	DISSOLVED OXYGEN CONCENTRATION/% OF THE FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	99.2	99.0	99.0	99.0	99.0
0.5	94.8	96.2	97.0	96.7	97.7
1.0	90.3	91.0	94.5	95.2	96.8
1.5	86.3	87.8	93.3	94.2	95.7
2.0	82.0	84.3	92.3	93.2	94.8
3.0	-	-	-	92.3	-
4.0	-	-	-	91.5	-
5.0	-	-	-	90.7	92.7
6.0	78.5	80.3	89.5	-	92.3
7.0	-	-	-	89.5	-
8.0	-	-	88.0	88.5	-
9.0	71.8	-	-	-	90.7
10.0	-	77.5	-	87.7	-
11.0	-	-	-	-	-
12.0	66.7	76.5	86.3	87.2	89.7

$$\sigma_n - 1 = 5.56 \times 10^{-3} \%$$

$$\sigma_n = 5.54 \times 10^{-3} \%$$

Appendix 37

Variation of dissolved oxygen concentration with time.

Pluronic L81P

TIME/HOURS	DISSOLVED OXYGEN CONCENTRATION/% IN THE FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	99.2	99.0	99.0	99.0	99.0
0.5	96.0	97.0	96.0	97.0	98.0
1.0	93.0	95.0	94.0	96.0	97.0
1.5	89.0	92.3	-	95.0	95.8
2.0	82.7	89.2	89.8	-	-
3.0	-	87.0	87.3	93.7	-
4.0	70.7	-	86.0	92.7	93.7
5.0	66.5	79.0	-	-	92.3
6.0	61.2	76.5	84.2	90.8	-
7.0	58.3	-	-	-	-
8.0	53.5	-	-	-	-
9.0	-	70.3	81.3	87.7	-
10.0	-	-	-	-	87.3
11.0	-	-	-	-	-
12.0	48.7	67.5	78.7	81.5	83.8

$$\sigma_{n-1} = 7.16 \times 10^{-3} \%$$

$$\sigma_{n} = 7.14 \times 10^{-3} \%$$

Appendix 38

Variation of dissolved oxygen concentration with time.

Pluronic L92P

TIME/HOURS	DISSOLVED OXYGEN CONCENTRATION/% IN THE FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	99.0	99.0	99.0	99.2	99.0
0.5	92.7	91.5	98.0	98.0	98.2
1.0	86.5	87.0	96.3	97.0	97.2
1.5	-	-	94.2	95.7	96.2
2.0	77.5	82.0	92.3	-	95.0
3.0	70.8	80.2	90.5	-	-
4.0	-	78.2	89.3	-	92.7
5.0	69.2	76.5	88.2	90.7	91.5
6.0	68.2	-	87.3	-	89.7
7.0	67.2	-	-	-	88.5
8.0	-	-	-	89.3	-
9.0	-	-	-	-	-
10.0	-	-	86.3	-	-
11.0	-	-	-	87.2	-
12.0	63.2	71.5	86.0	86.3	86.3

$\sigma_{n-1} = 6.04 \times 10^{-3}$ %

$\sigma_{n} = 6.02 \times 10^{-3}$ %

Appendix 39

Variation of dissolved oxygen concentration with time.

Pluronic L101P

TIME/HOURS	DISSOLVED OXYGEN CONCENTRATION/% IN THE FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	99.0	99.0	99.0	99.0	99.0
0.5	91.8	98.0	98.0	98.2	98.0
1.0	88.3	96.3	-	97.3	97.2
1.5	-	94.7	96.2	96.3	96.5
2.0	84.3	92.7	94.0	95.3	96.0
3.0	81.5	90.7	-	94.5	95.5
4.0	-	88.7	92.3	93.8	95.0
5.0	77.7	-	-	-	-
6.0	76.3	-	90.2	93.0	94.5
7.0	74.5	-	-	-	-
8.0	72.8	80.7	-	-	94.0
9.0	-	-	88.0	91.7	-
10.0	-	-	-	-	-
11.0	67.5	-	-	-	-
12.0	66.3	76.8	86.0	89.0	92.3

$$\sigma_{n-1} = 5.23 \times 10^{-3} \%$$

$$\sigma_{n} = 5.21 \times 10^{-3} \%$$

Appendix 40

Variation of dissolved oxygen concentration with time.

Plronic L121P

TIME/HOURS	DISSOLVED OXYGEN CONCENTRATION/% IN THE FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	99.0	99.0	99.0	99.0	99.0
0.5	93.0	96.2	97.0	97.0	98.0
1.0	86.8	91.8	95.0	95.5	97.0
1.5	83.5	90.2	92.7	93.8	96.0
2.0	81.2	88.5	91.0	92.0	95.0
3.0	-	-	89.8	-	-
4.0	78.7	-	88.8	90.5	93.8
5.0	-	-	-	-	-
6.0	77.2	-	87.7	89.3	92.8
7.0	-	85.2	-	-	-
8.0	-	-	-	88.3	91.5
9.0	-	83.0	86.7	-	-
10.0	74.7	-	-	-	90.0
11.0	-	-	-	-	-
12.0	73.7	81.2	84.8	87.0	88.7

$$\bar{\sigma}_n - 1 = 4.63 \times 10^{-3} \%$$

$$\bar{\sigma}_n = 4.61 \times 10^{-3} \%$$

Appendix 41

Variation of dissolved oxygen concentration with time.

No Polymer Present

TIME/HOURS	DISSOLVED OXYGEN CONCENTRATION/% IN FERMENTATION MEDIUM
0.0	99.0
0.5	95.8
1.0	88.2
1.5	81.8
2.0	78.8
3.0	-
4.0	-
5.0	72.6
6.0	-
7.0	-
8.0	63.5
9.0	62.0
10.0	-
11.0	-
12.0	58.0

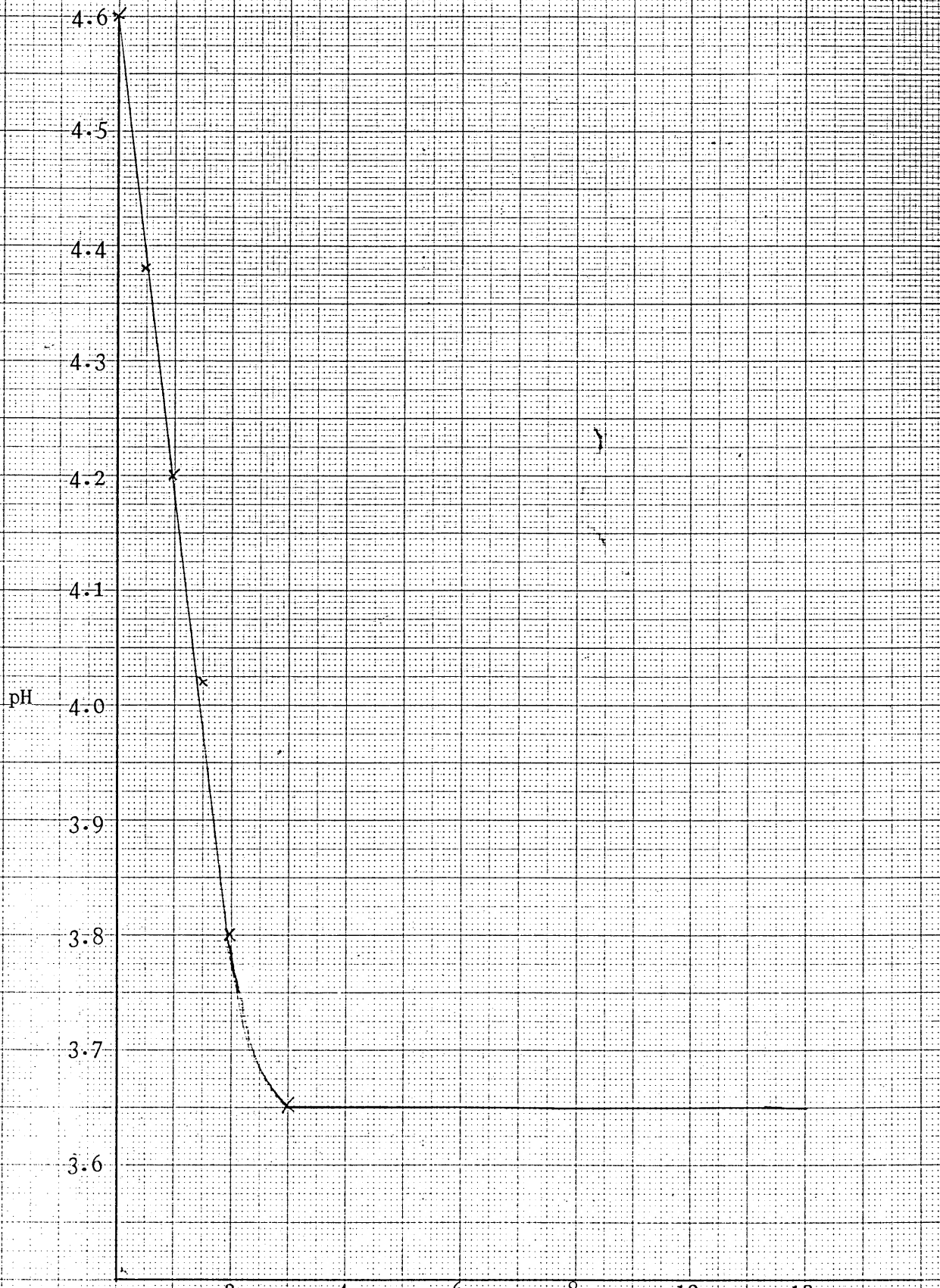
$$\sigma_{n-1} = 6.45 \times 10^{-3} \%$$

$$\sigma_n = 6.33 \times 10^{-3} \%$$

Appendix 42

No Polymer

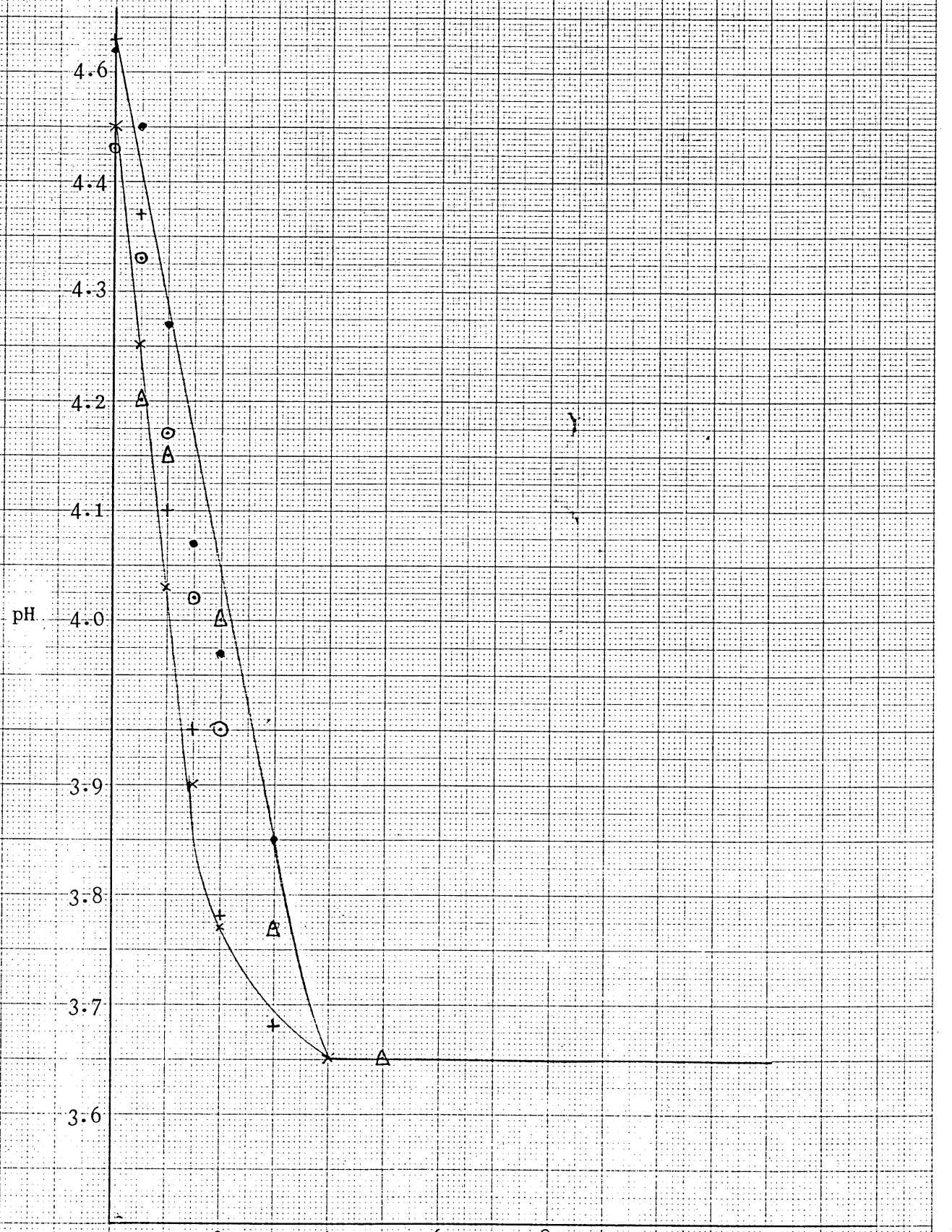
Graph of pH against time



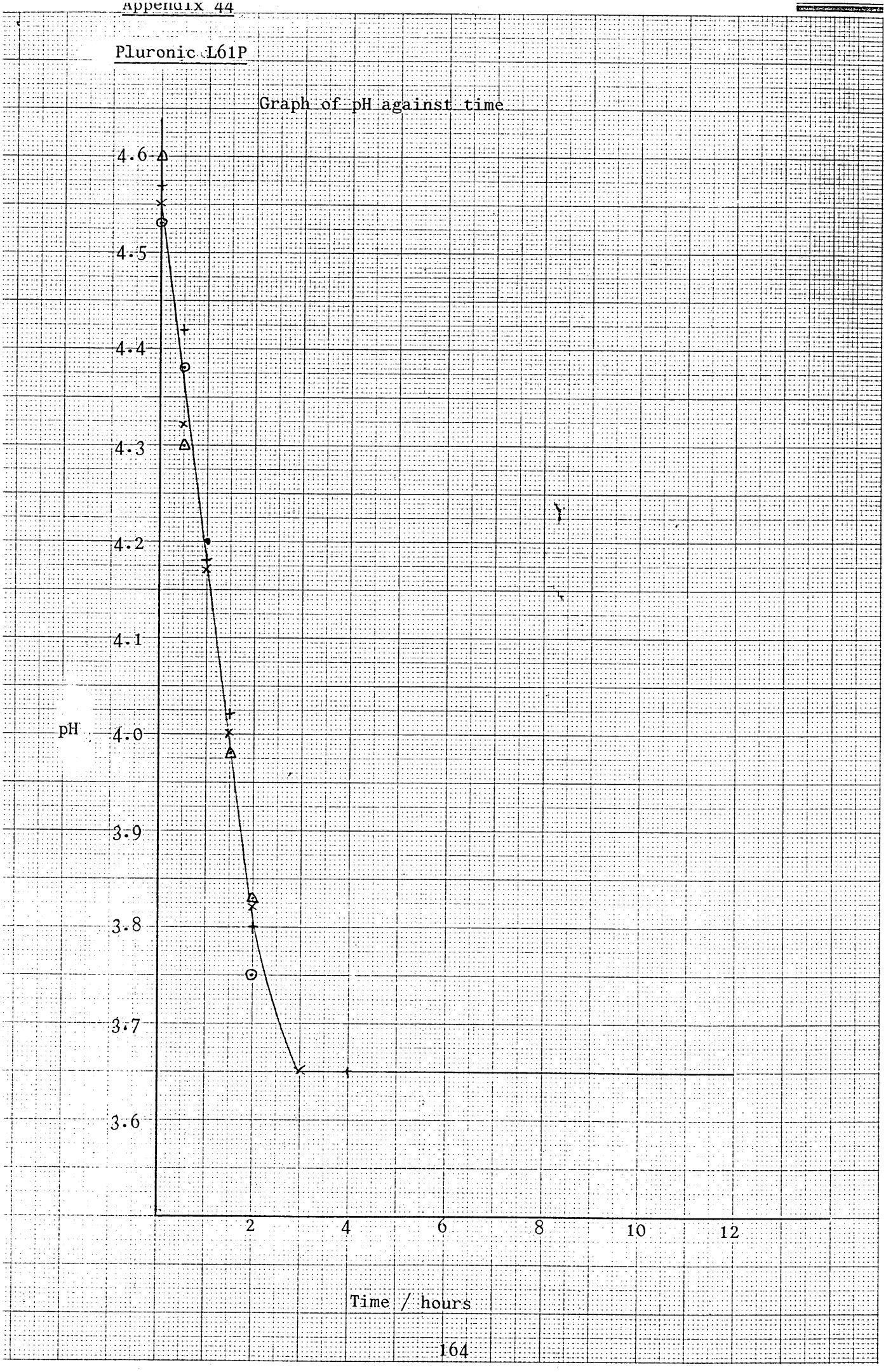
Appendix 43

Pluronic L31P

Graph of pH against time



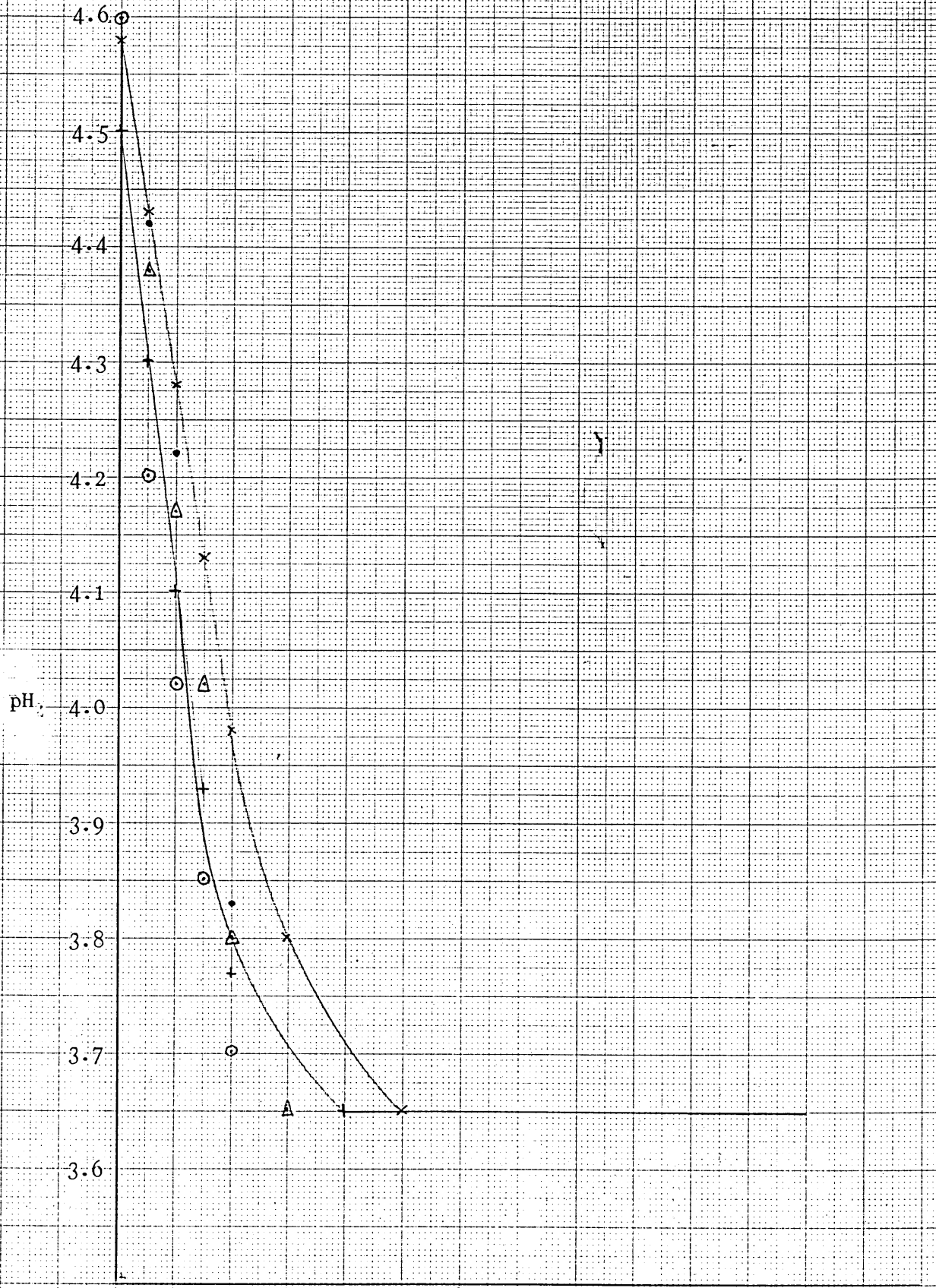
Pluronic L61P



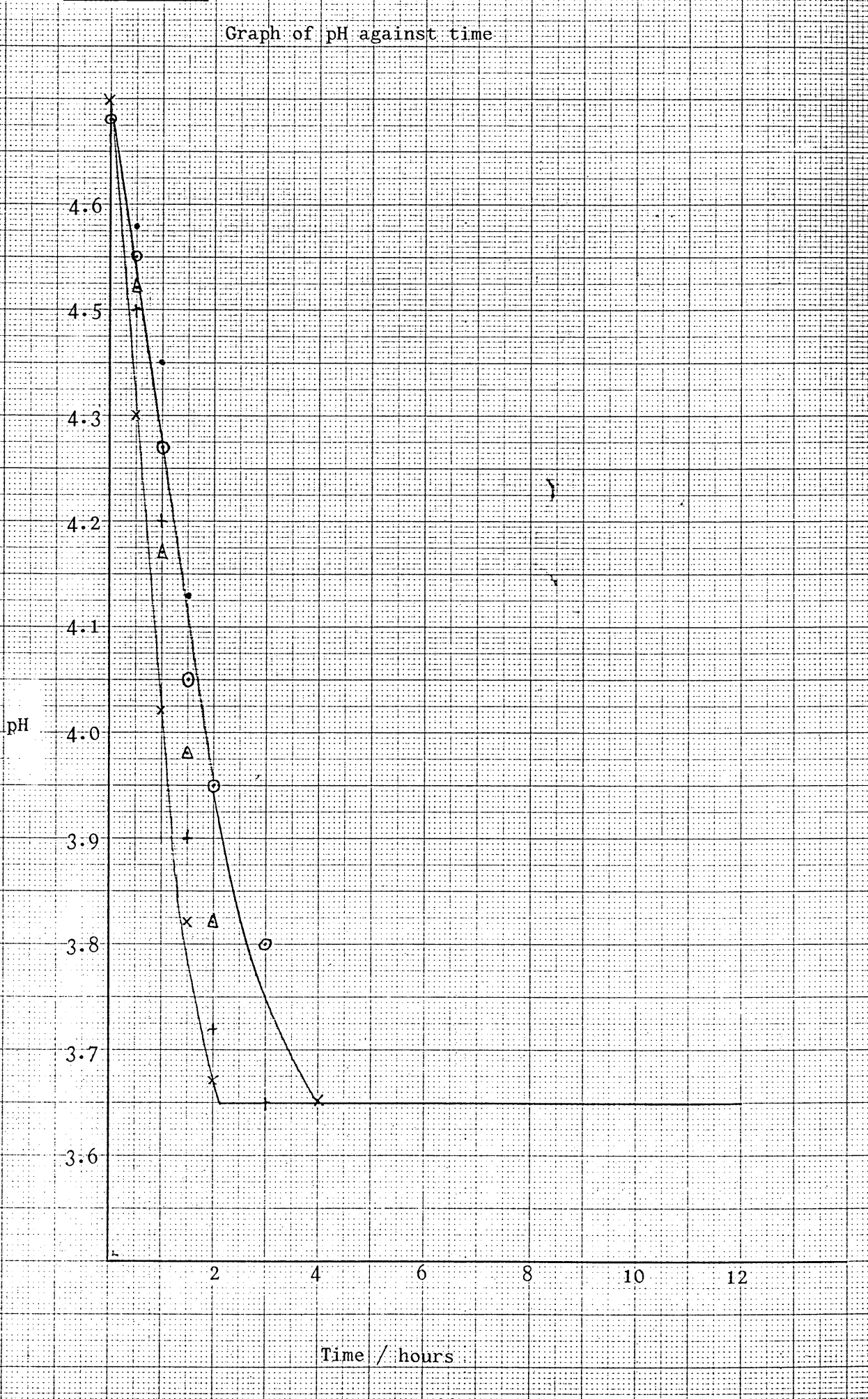
Appendix 45

Pluronic L62P

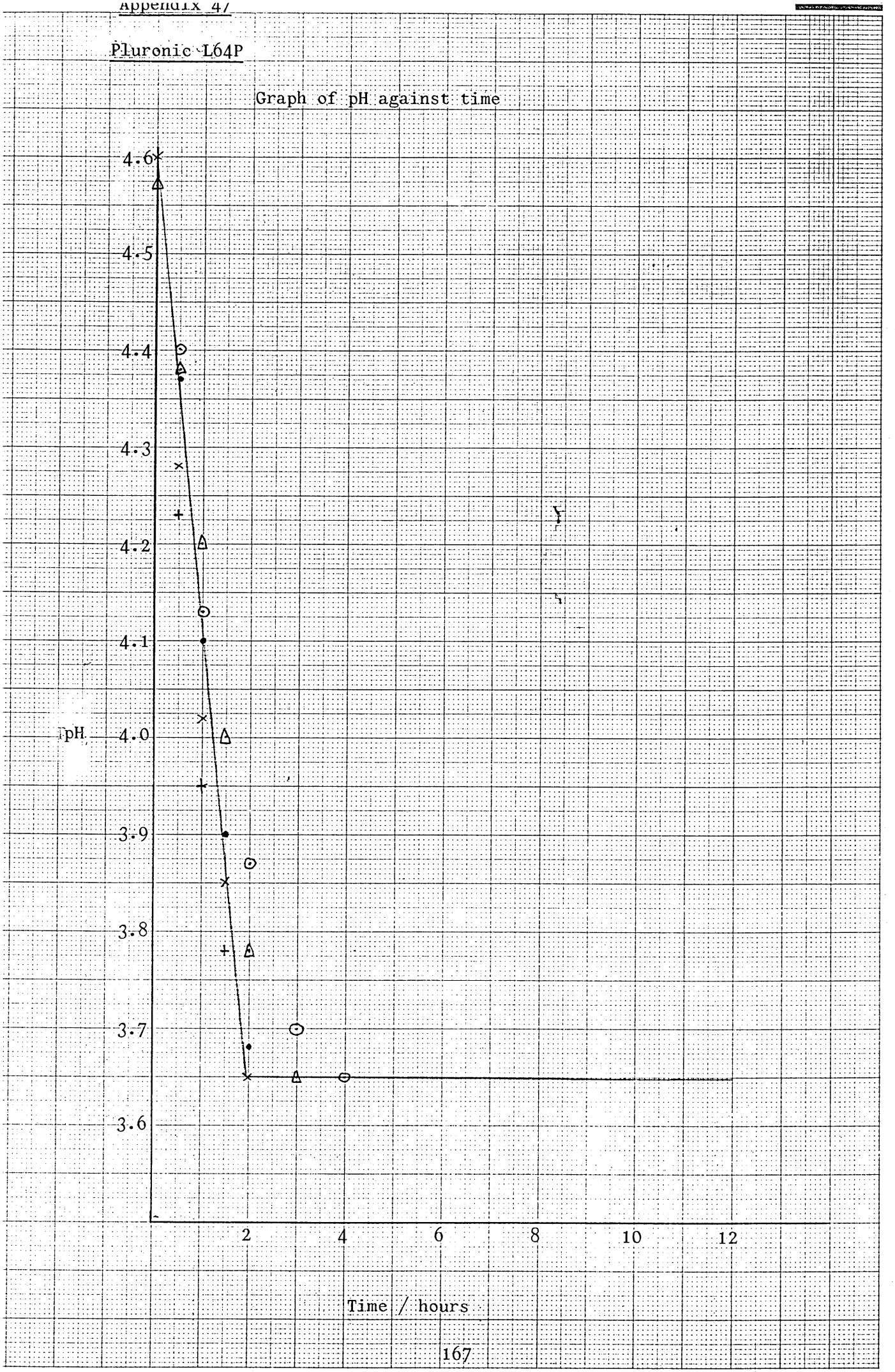
Graph of pH against time



Pluronic L63P

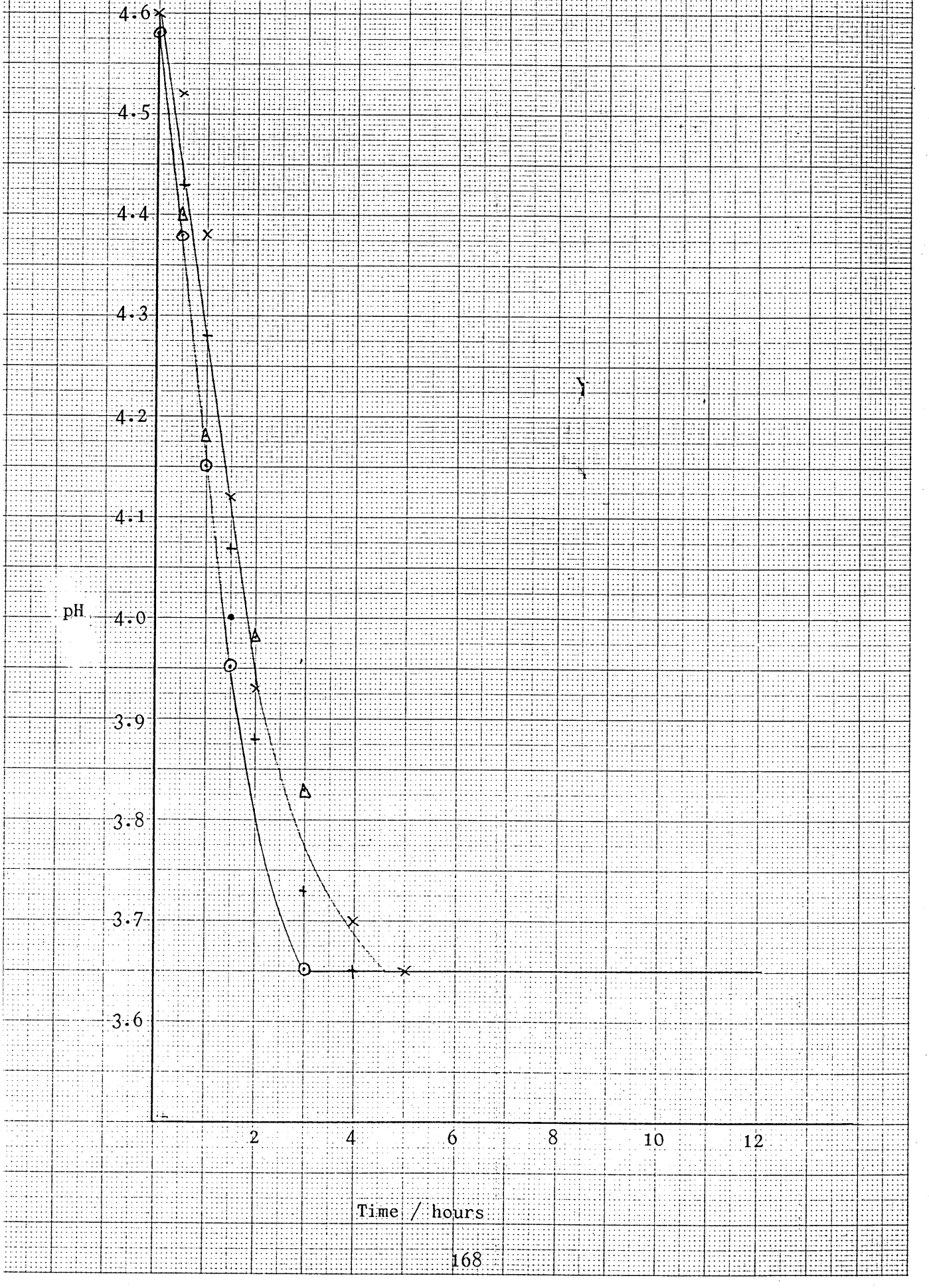


Pluronic L64P



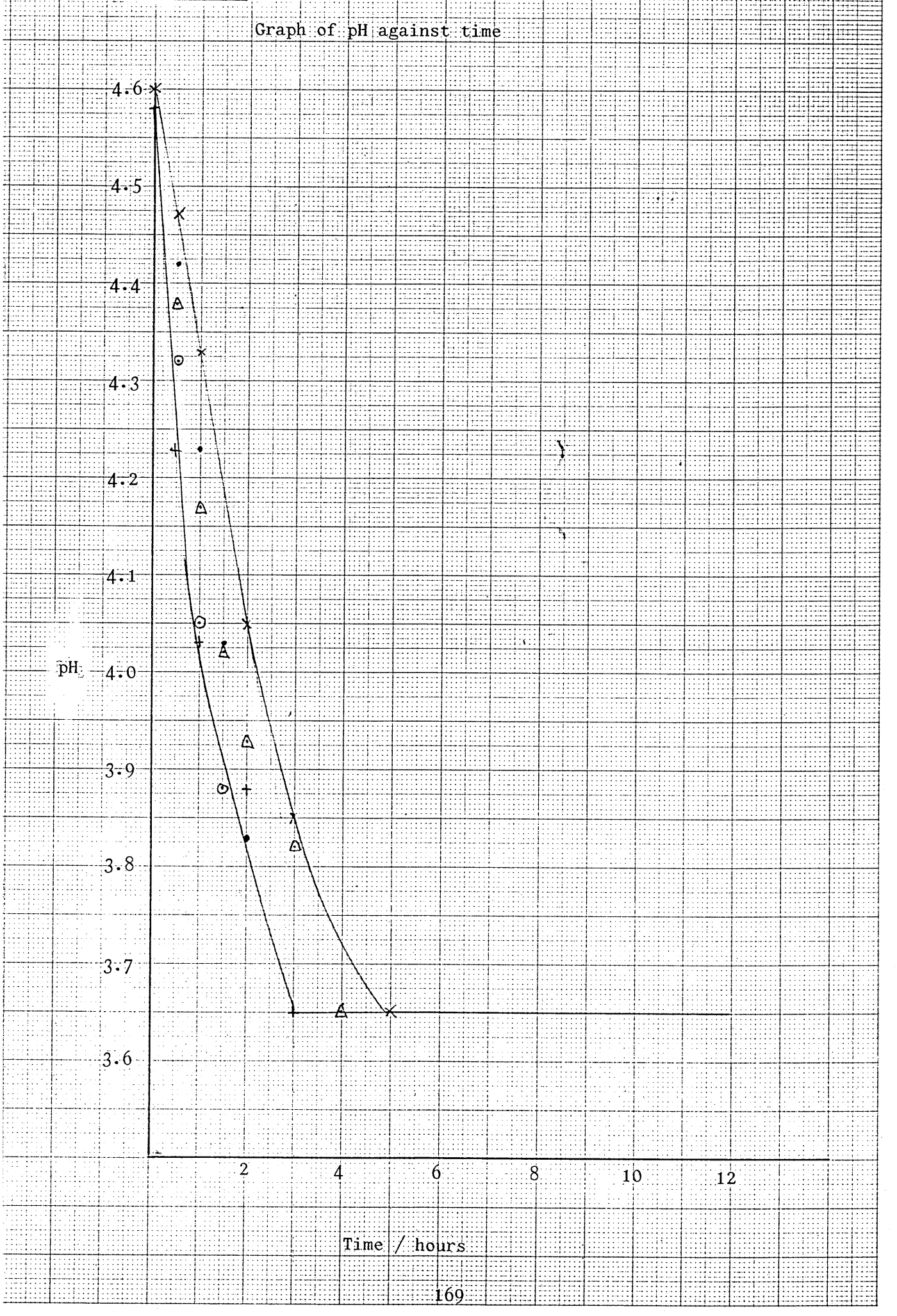
Pluronic L81P

Graph of pH against time



Appendix 49

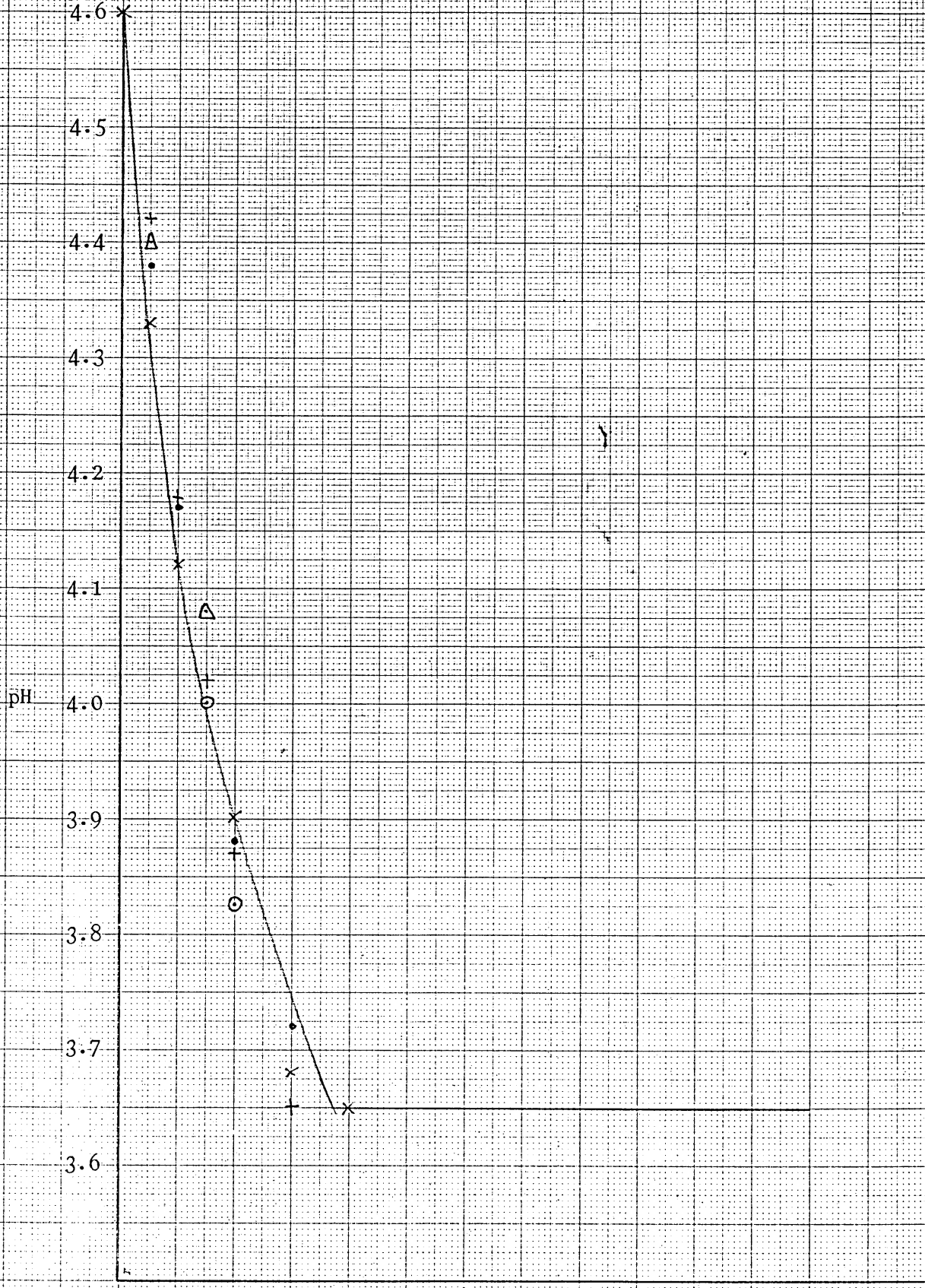
Pluronic L92P



Appendix 50

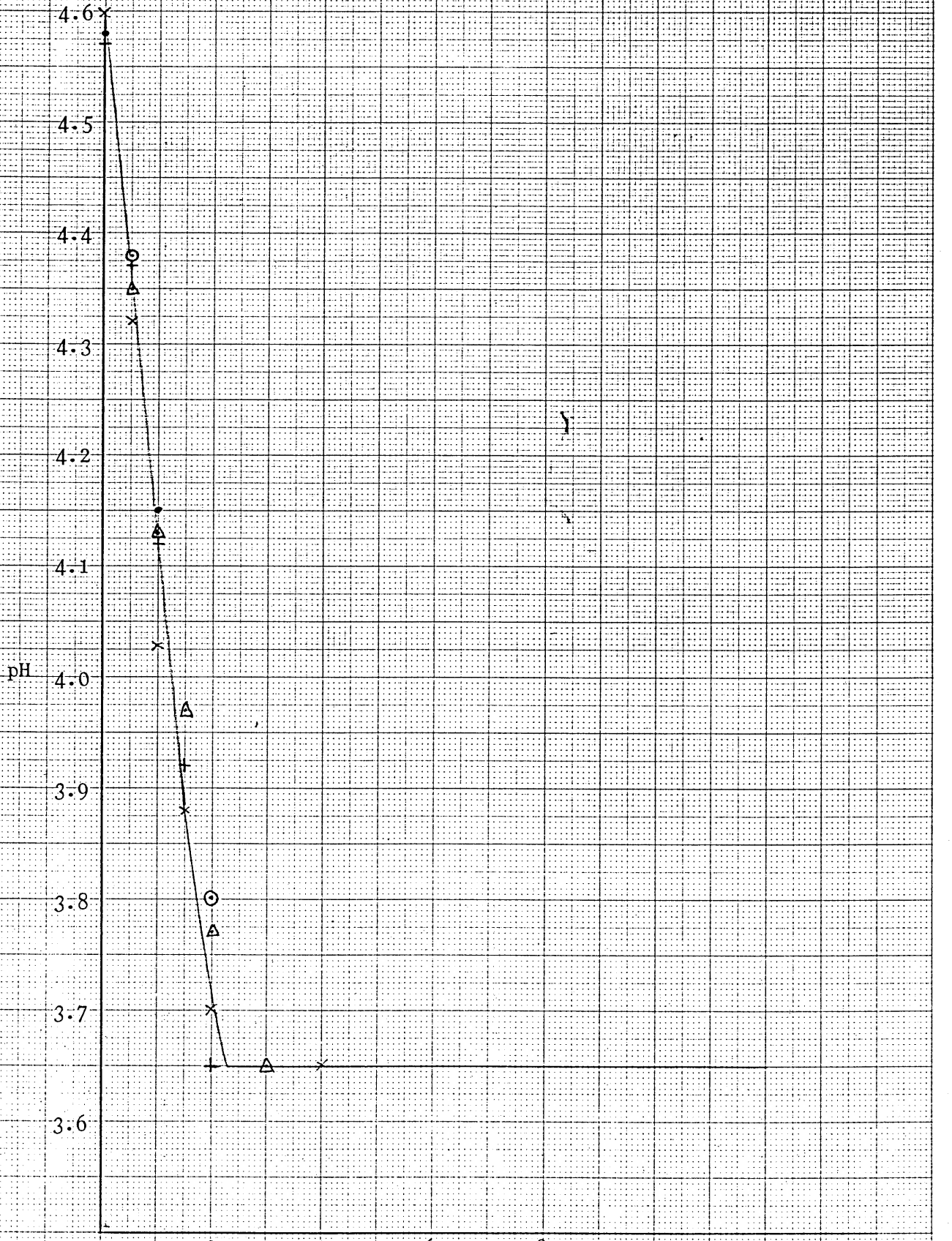
Pluronic L101P

Graph of pH against time



Pluronic L121P

Graph of pH against time



Appendix 52

Variation of pH with time

NB: When pH falls to 3.65 all subsequent readings for that fermentation were artificially maintained at 3.65.

No Polymer Present

TIME/HOURS	pH OF FERMENTATION MEDIUM
0.0	4.60
0.5	4.38
1.0	4.20
1.5	4.02
2.0	3.80
3.0	3.65
4.0	-
5.0	-
6.0	-
7.0	-
8.0	-
9.0	-
10.0	-
11.0	-
12.0	-

$$\sigma_n - 1 = 3.38 \times 10^{-3}$$

$$\sigma_n = 3.28 \times 10^{-3}$$

Appendix 53

Variation of pH with time

Pluronic L31P

TIME/HOURS	pH OF FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	4.50	4.58	4.50	4.48	4.57
0.5	4.30	4.42	4.25	4.38	4.50
1.0	4.08	4.15	4.20	4.22	4.32
1.5	3.90	3.95	-	4.07	4.12
2.0	3.77	3.78	4.05	3.95	4.02
3.0	-	3.68	3.77	3.77	3.85
4.0	3.65	3.65	-	-	3.65
5.0			3.65	3.65	
6.0					
7.0					
8.0					
9.0					
10.0					
11.0					
12.0					

$$\sigma_n - 1 = 5.95 \times 10^{-3}$$

$$\sigma_n = 5.92 \times 10^{-3}$$

Appendix 54

Variation of pH with time

Pluronic L61P

TIME/HOURS	pH OF FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	4.55	4.58	4.60	4.53	4.60
0.5	4.32	4.42	4.30	4.38	4.38
1.0	4.17	4.18	4.17	4.18	4.20
1.5	4.00	4.02	3.98	4.02	4.00
2.0	3.82	3.80	3.83	3.75	3.82
3.0	3.65	-	-	-	3.65
4.0		3.65	3.65	3.65	
5.0					
6.0					
7.0					
8.0					
9.0					
10.0					
11.0					

$$\sigma_{n-1} = 6.97 \times 10^{-3}$$

$$\sigma_{n} = 6.93 \times 10^{-3}$$

Appendix 55

Variation of pH with time

Pluronic L62P

TIME/HOURS	pH OF FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	4.58	4.50	4.58	4.60	4.60
0.5	4.43	4.30	4.38	4.20	4.42
1.0	4.28	4.10	4.17	4.02	4.22
1.5	4.13	3.93	4.02	3.85	4.02
2.0	3.98	3.77	3.80	3.70	3.83
3.0	3.80	-	3.65	3.65	-
4.0	-	3.65			3.65
5.0	3.65				
6.0					
7.0					
8.0					
9.0					
10.0					
11.0					
12.0					

$$\sigma_n - 1 = 9.47 \times 10^{-3}$$

$$\sigma_n = 9.42 \times 10^{-3}$$

Appendix 56

Variation of pH with time

Pluronic L63P

TIME/HOURS	pH OF FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	4.60	4.60	4.60	4.58	4.60
0.5	4.30	4.40	4.42	4.45	4.48
1.0	4.02	4.20	4.17	4.27	4.35
1.5	3.82	3.90	3.98	4.05	4.13
2.0	3.67	3.72	3.82	3.95	3.95
3.0	-	3.65	3.65	3.80	-
4.0	3.65			3.65	3.65
5.0					
6.0					
7.0					
8.0					
9.0					
10.0					
11.0					
12.0					

$$\sigma_{n-1} = 8.08 \times 10^{-3}$$

$$\sigma_n = 8.04 \times 10^{-3}$$

Appendix 57

Variation of pH with time

Pluronic L64P

TIME/HOURS	pH OF FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	4.60	4.60	4.57	4.60	4.58
0.5	4.28	4.23	4.38	4.40	4.37
1.0	4.02	3.95	4.20	4.13	4.10
1.5	3.85	3.78	4.00	4.00	3.90
2.0	3.65	3.65	3.78	3.87	3.68
3.0			3.65	3.70	3.65
4.0				3.65	
5.0					
6.0					
7.0					
8.0					
9.0					
10.0					
11.0					
12.0					

$$\sigma_{n-1} = 9.20 \times 10^{-3}$$

$$\sigma_{n} = 9.15 \times 10^{-3}$$

Appendix 58

Variation of pH with time

Pluronic L81P

TIME/HOURS	pH OF FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	4.60	4.60	4.60	4.58	4.60
0.5	4.52	4.43	4.40	4.38	4.40
1.0	4.38	4.28	4.18	4.15	4.18
1.5	4.12	4.07	-	3.95	4.00
2.0	3.93	3.88	3.98	-	-
3.0	-	3.73	3.83	3.65	-
4.0	3.70	3.65	3.65		3.65
5.0	3.65				
6.0					
7.0					
8.0					
9.0					
10.0					
11.0					
12.0					

$$\sigma_n - 1 = 9.51 \times 10^{-3}$$

$$\sigma_n = 9.46 \times 10^{-3}$$

Appendix 59

Variation of pH with time

Pluronic L92P

TIME/HOURS	pH OF FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	4.60	4.58	4.58	4.60	4.60
0.5	4.47	4.23	4.38	4.32	4.42
1.0	4.33	4.03	4.17	4.05	4.23
1.5	-	-	4.02	3.87	4.03
2.0	4.05	3.87	3.93	-	3.83
3.0	3.85	3.65	3.82	-	-
4.0	-		3.65	-	3.65
5.0	3.65			3.65	
6.0					
7.0					
8.0					
9.0					
10.0					
11.0					
12.0					

$$\sigma_{n-1} = 8.73 \times 10^{-3}$$

$$\sigma_{n} = 8.68 \times 10^{-3}$$

Appendix 60

Variation of pH with time

Pluronic L101P

TIME/HOURS	pH OF FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	4.60	4.60	4.60	4.60	4.60
0.5	4.33	4.42	4.40	4.40	4.38
1.0	4.12	4.18	-	4.18	4.17
1.5	-	4.02	4.08	4.00	4.02
2.0	3.90	3.87	3.87	3.85	3.88
3.0	3.68	3.65	-	3.68	3.72
4.0	-		3.65	3.65	3.65
5.0	3.65				
6.0					
7.0					
8.0					
9.0					
10.0					
11.0					
12.0					

$\sigma_n - 1 = 8.64 \times 10^{-3}$

$\sigma_n = 8.60 \times 10^{-3}$

Appendix 61

Variation of pH with time

Pluronic L121P

TIME/HOURS	pH OF FERMENTATION MEDIUM USING THE FOLLOWING POLYMER WEIGHT / mg				
	5	15	50	75	100
0.0	4.60	4.57	4.60	4.57	4.58
0.5	4.32	4.37	4.35	4.38	4.38
1.0	4.03	4.12	4.13	4.13	4.15
1.5	3.88	3.92	3.97	3.97	3.97
2.0	3.70	3.65	3.77	3.80	3.77
3.0	-		3.65	-	-
4.0	3.65			3.65	3.65
5.0					
6.0					
7.0					
8.0					
9.0					
10.0					
11.0					
12.0					

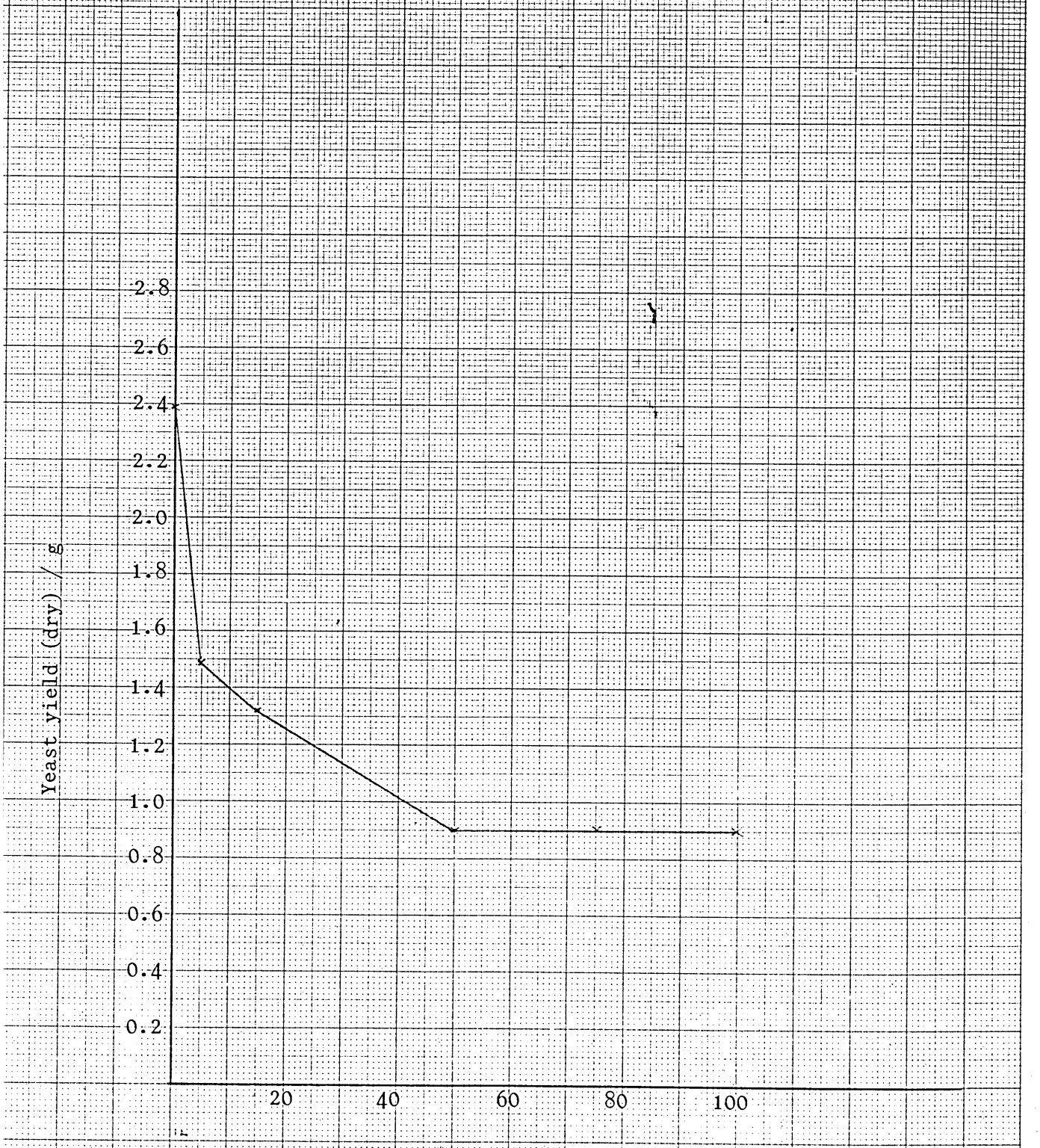
$$\sigma_n - 1 = 9.33 \times 10^{-3}$$

$$\sigma_n = 9.27 \times 10^{-3}$$

Appendix 62

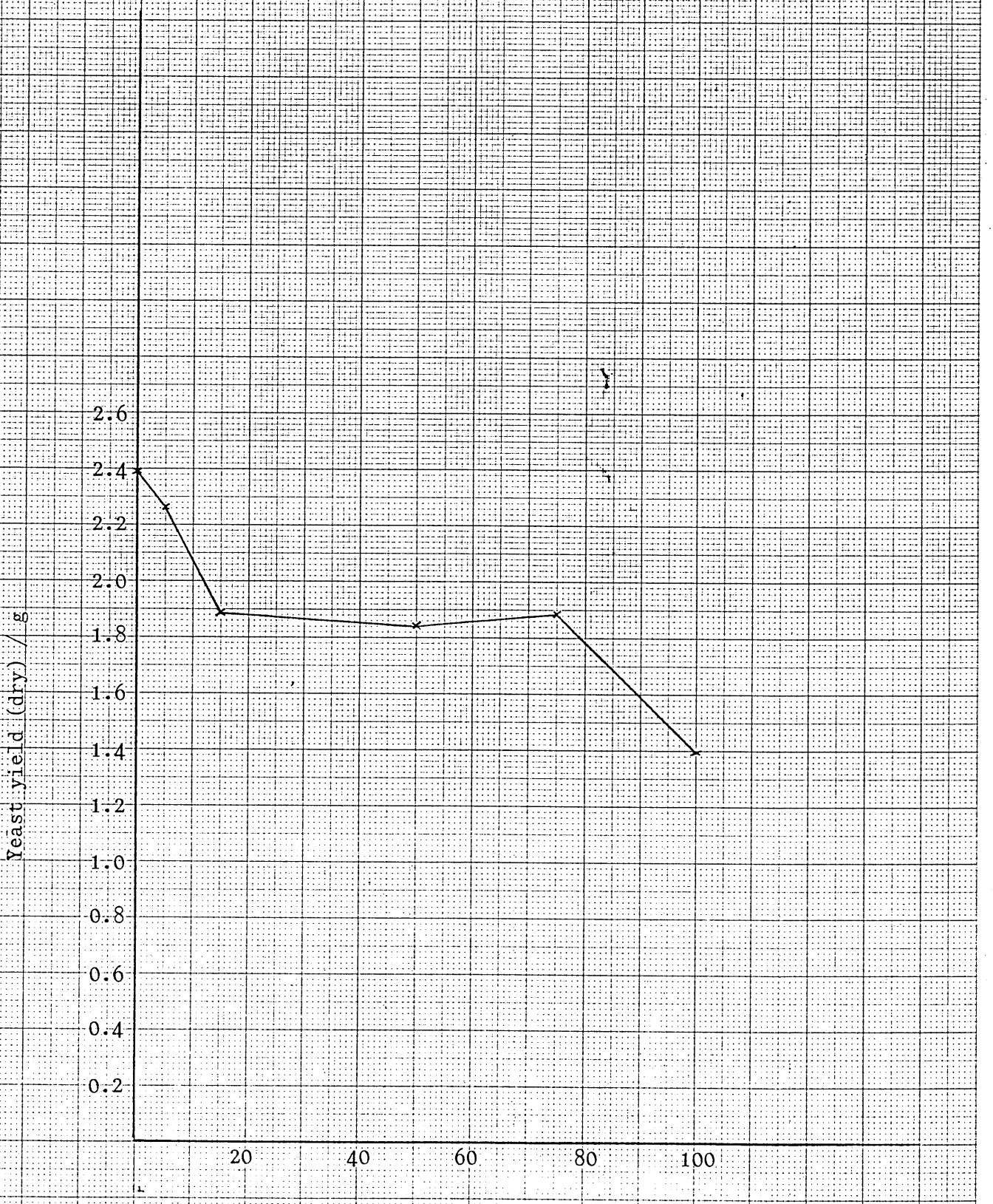
Pluronic L31P

Graph of yeast yield against polymer weight



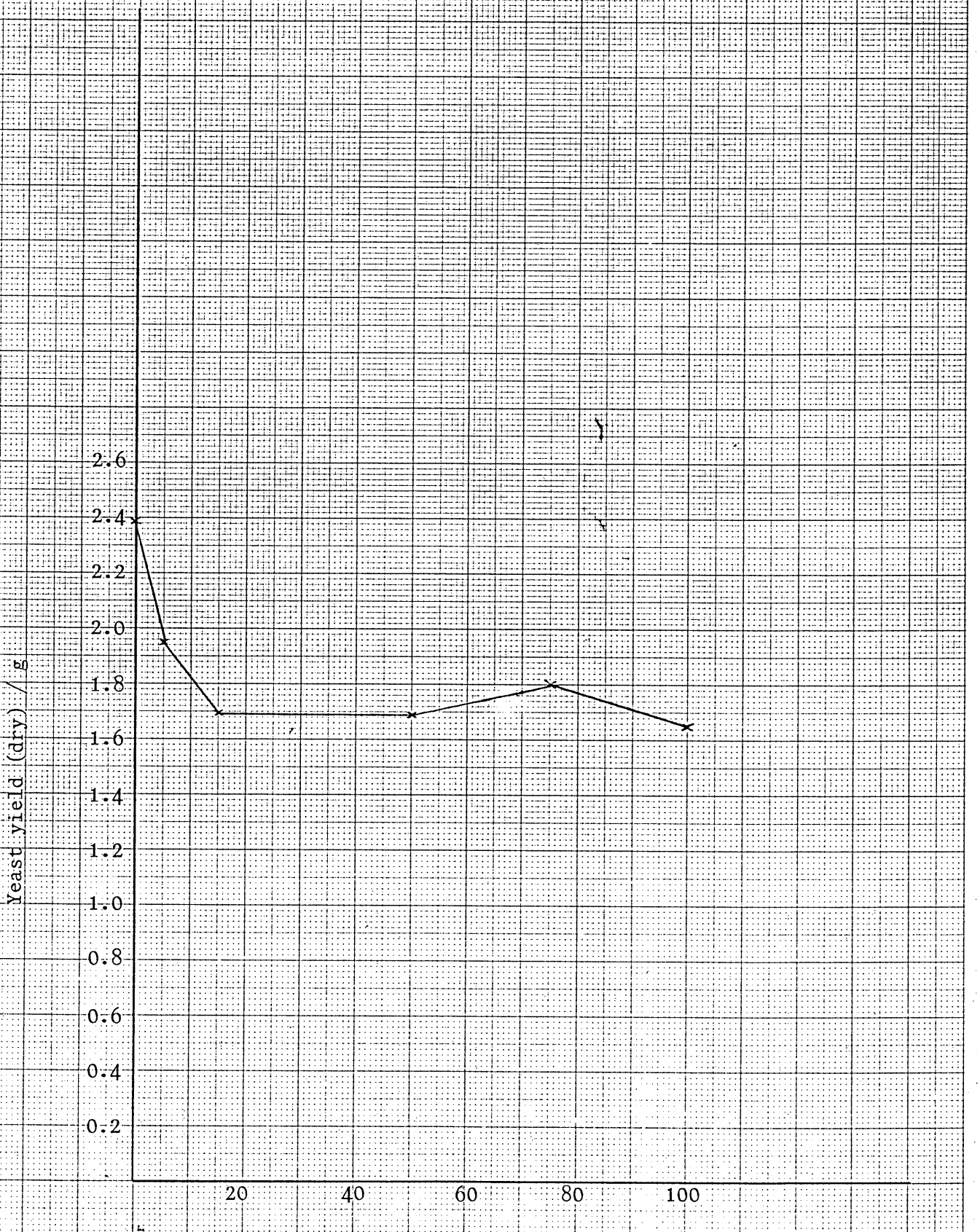
Pluronic L61P

Graph of yeast yield against polymer weight



Pluronic L62P

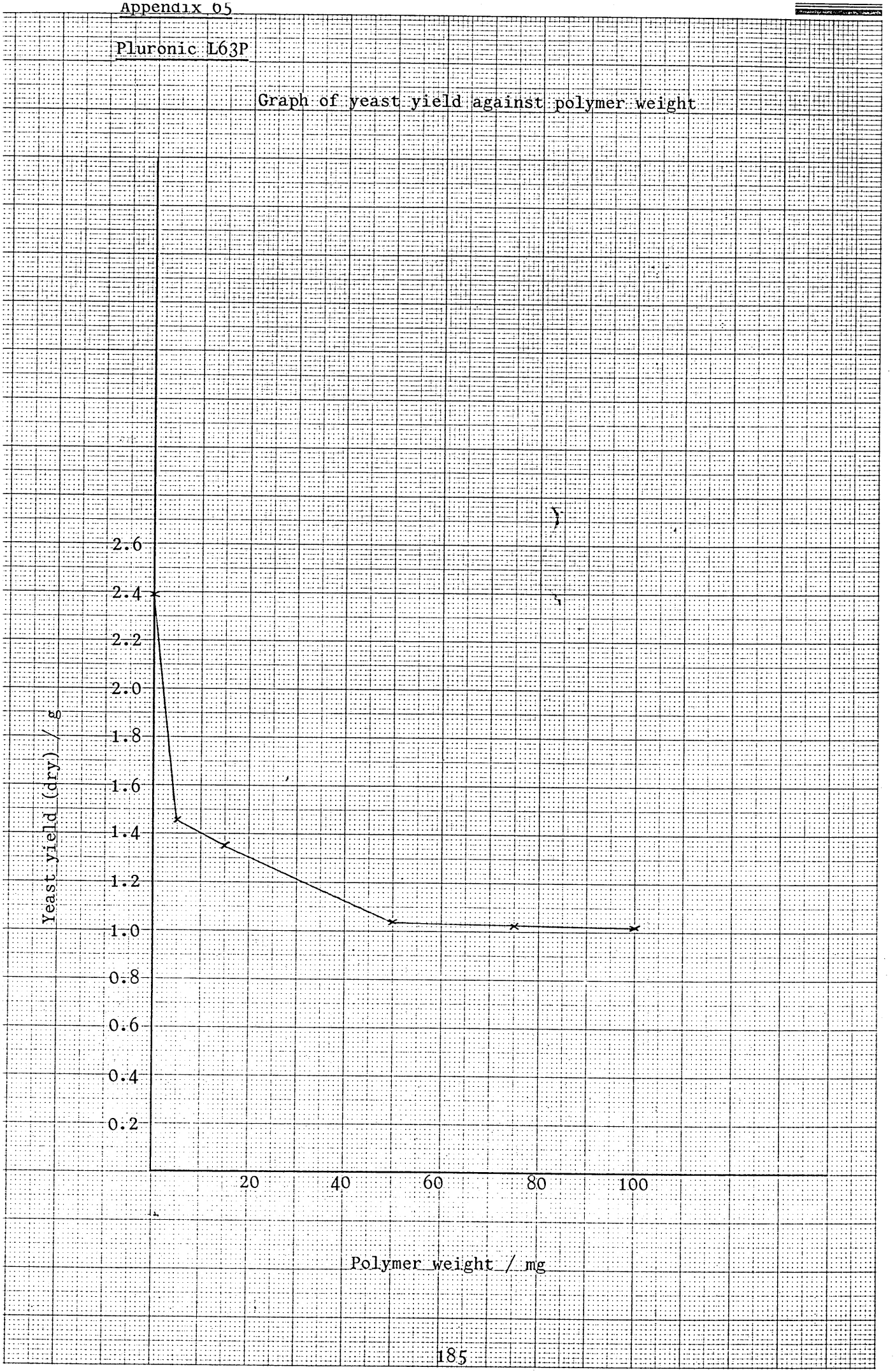
Graph of yeast yield against polymer weight



Appendix 05

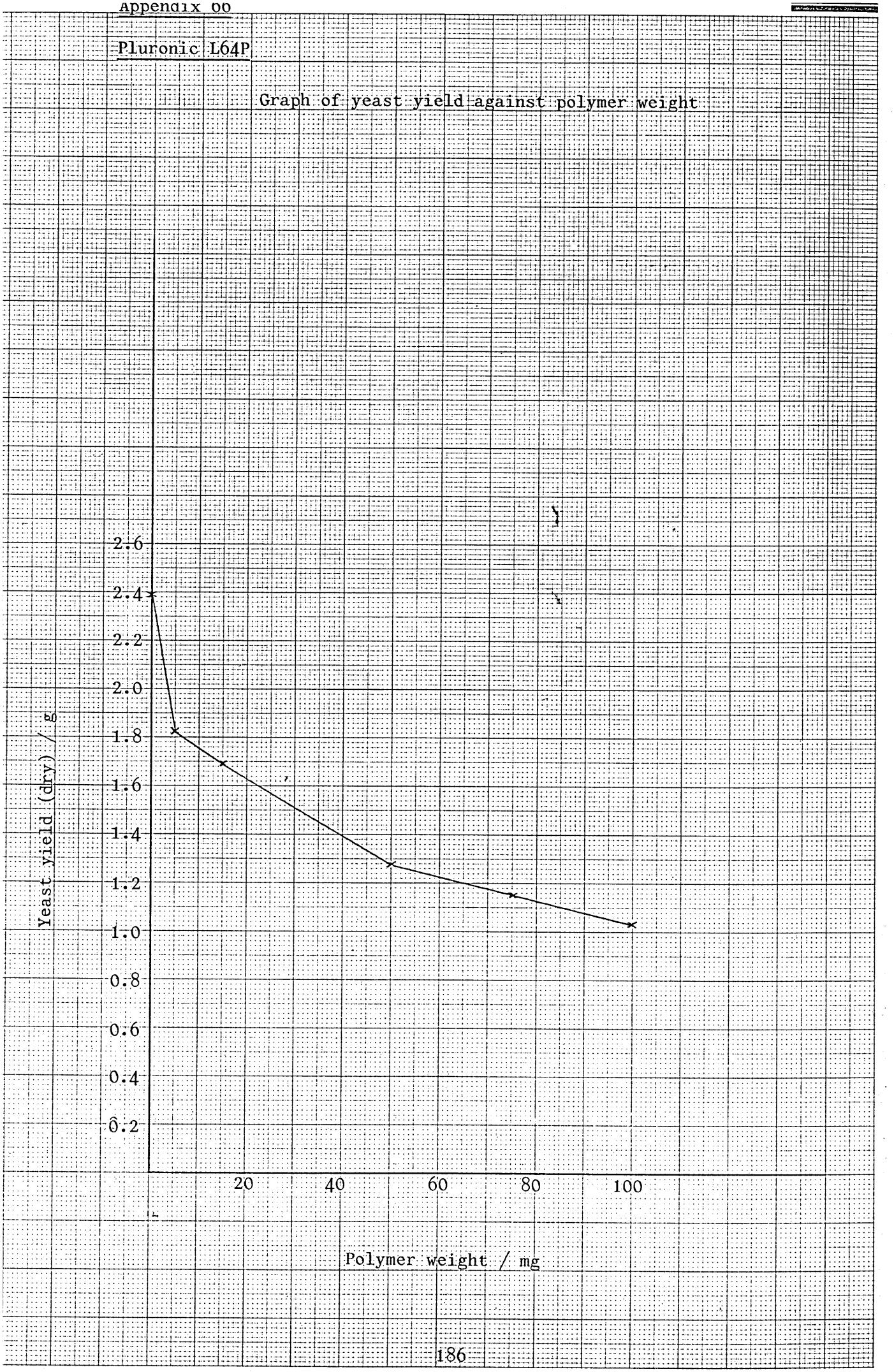
Pluronic L63P

Graph of yeast yield against polymer weight



Pluronic L64P

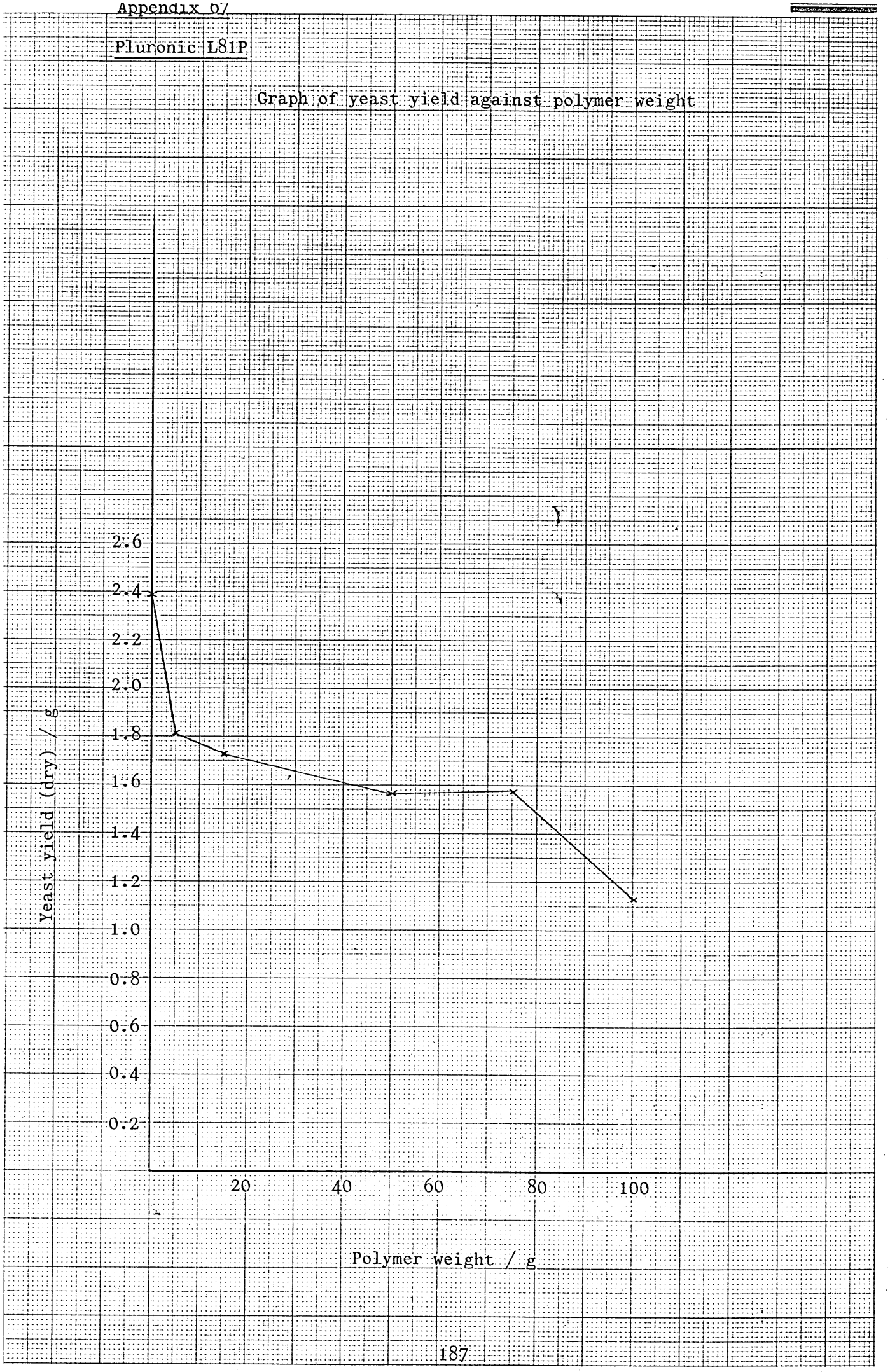
Graph of yeast yield against polymer weight



Appendix 67

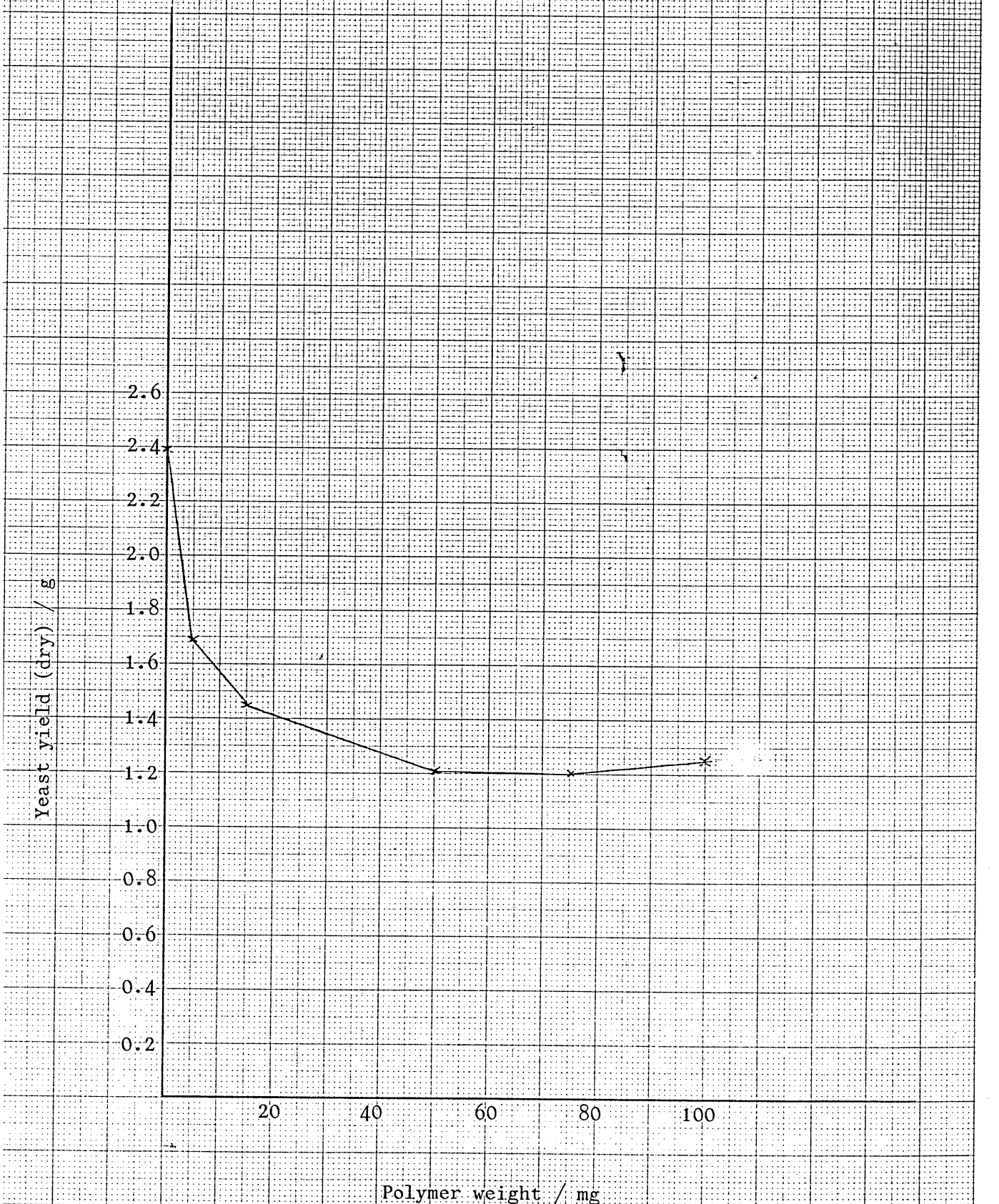
Pluronic L81P

Graph of yeast yield against polymer weight



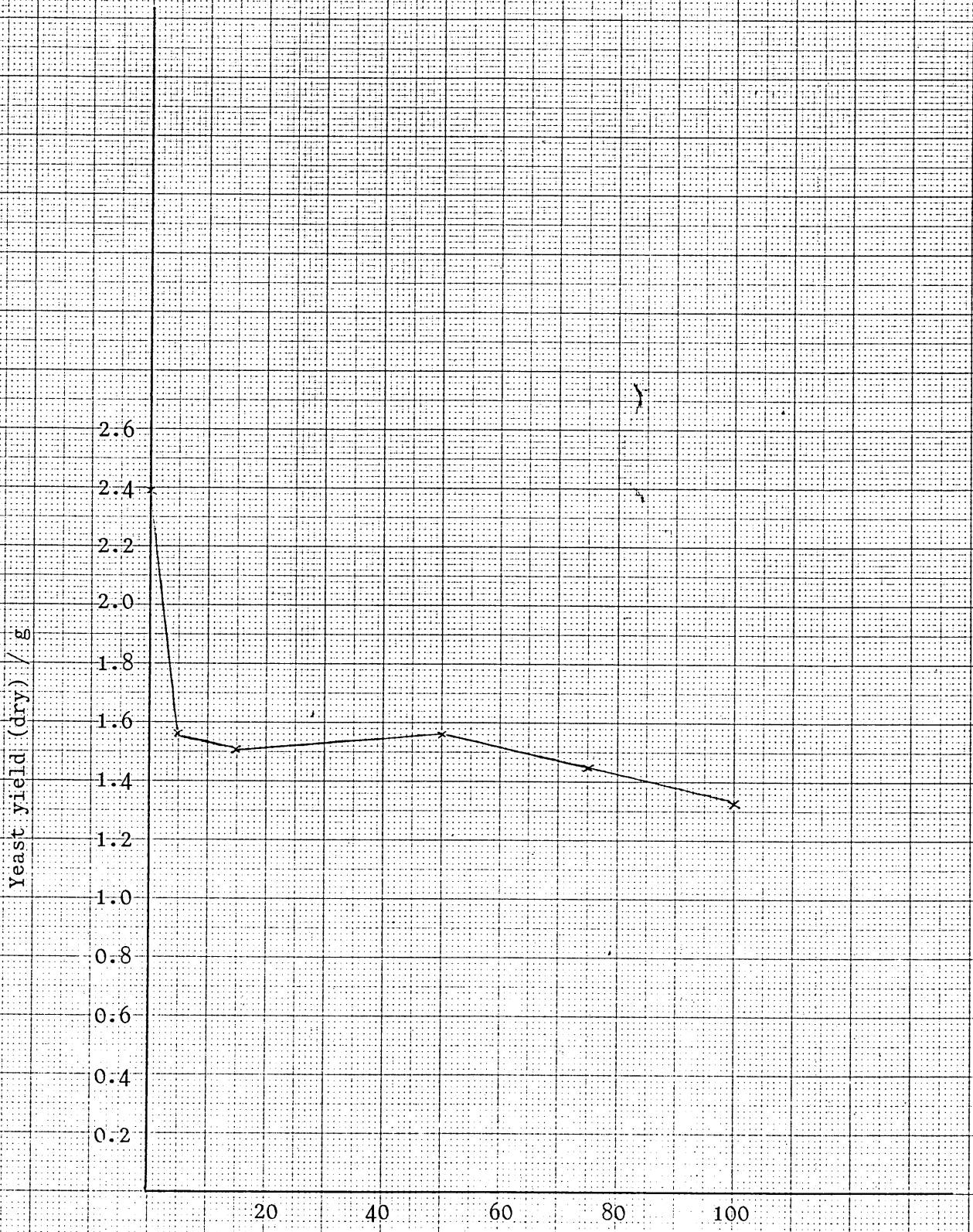
Pluronic L92P

Graph of yeast yield against polymer weight



Pluronic L101P

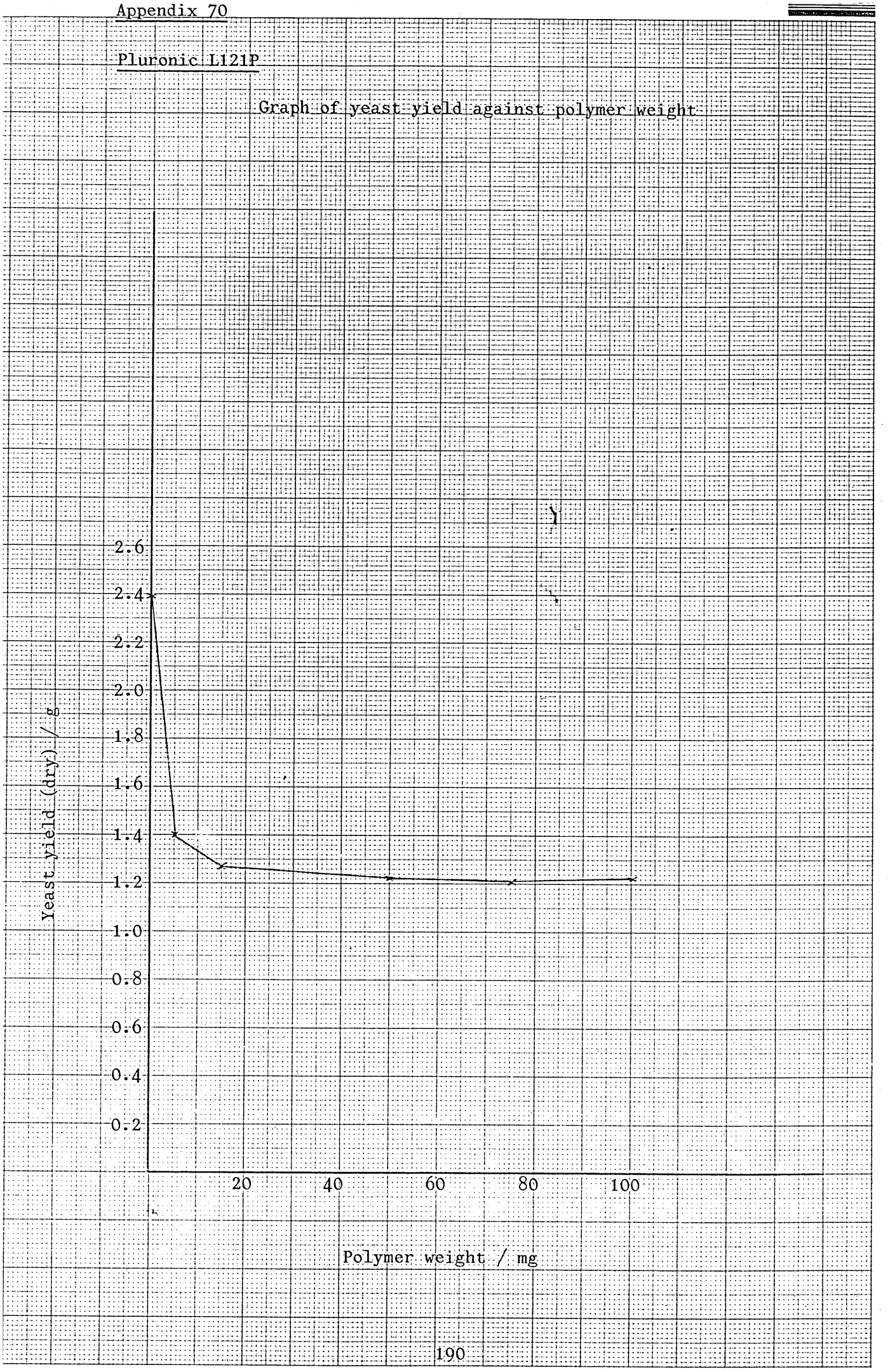
Graph of yeast yield against polymer weight



Appendix 70

Pluronic L121P

Graph of yeast yield against polymer weight



Appendix 71

Variation of yeast yield with polymer weight

Pluronic L31P

POLYMER WEIGHT / mg	YEAST YIELD (DRY) / g
5	1.484
15	1.319
50	0.904
75	0.915
100	0.909

$\sigma_{n-1} = 7.57 \times 10^{-3}$ g

$\sigma_{n} = 7.31 \times 10^{-3}$ g

Appendix 72

Variation of yeast yield with polymer weight

Pluronic L61P

POLYMER WEIGHT / mg	YEAST YIELD (DRY) / g
5	2.253
15	1.890
50	1.842
75	1.886
100	1.398

$\sigma_{n-1} = 13.50 \times 10^{-3}$ g

$\sigma_n = 13.04 \times 10^{-3}$ g

Appendix 73

Variation of yeast yield with polymer weight

Pluronic L62P

POLYMER WEIGHT / mg	YEAST YIELD (DRY) / g
5	1.958
15	1.690
50	1.693
75	1.803
100	1.649

$\sigma_{n-1} = 14.65 \times 10^{-3}$ g

$\sigma_{n} = 14.16 \times 10^{-3}$ g

Appendix 74

Variation of yeast yield with polymer weight

Pluronic L63P

POLYMER WEIGHT / g	YEAST YIELD (DRY) / g
5	1.459
15	1.358
50	1.033
75	1.035
100	1.029

$$\sigma_{n-1} = 6.11 \times 10^{-3} \text{ g}$$

$$\sigma_{n} = 5.90 \times 10^{-3} \text{ g}$$

Appendix 75

Variation of yeast yield with polymer weight

Pluronic L64P

POLYMER WEIGHT / mg	YEAST YIELD (DRY) / g
5	1.828
15	1.696
50	1.272
75	1.142
100	1.026

$\sigma_{n-1} = 19.08 \times 10^{-3}$ g

$\sigma_{n} = 18.44 \times 10^{-3}$ g

Appendix 76

Variation of yeast yield with polymer weight

Pluronic L81P

POLYMER WEIGHT / mg	YEAST YIELD (DRY) / g
5	1.820
15	1.731
50	1.567
75	1.582
100	1.121

$$\sigma_{n-1} = 12.21 \times 10^{-3} \text{ g}$$

$$\sigma_n = 11.79 \times 10^{-3} \text{ g}$$

Appendix 77

Variation of yeast yield with polymer weight

Pluronic L92P

POLYMER WEIGHT / mg	YEAST YIELD (DRY) / g
5	1.650
15	1.451
50	1.212
75	1.194
100	1.272

$$\sigma_{n-1} = 11.13 \times 10^{-3} \text{ g}$$

$$\sigma_{n} = 10.76 \times 10^{-3} \text{ g}$$

Appendix 78

Variation of yeast yield with polymer weight

Pluronic L101P

POLYMER WEIGHT / mg	YEAST YIELD (DRY) / g
5	1.570
15	1.513
50	1.570
75	1.456
100	1.313

$$\sigma_{n-1} = 16.90 \times 10^{-3} \text{ g}$$

$$\sigma_{n} = 16.33 \times 10^{-3} \text{ g}$$

Appendix 79

Variation of yeast yield with polymer weight

Pluronic L121P

POLYMER WEIGHT / mg	YEAST YIELD (DRY) / g
5	1.400
15	1.274
50	1.222
75	1.208
100	1.229

$$\sigma_{n-1} = 14.87 \times 10^{-3} \text{ g}$$

$$\sigma_{n} = 14.36 \times 10^{-3} \text{ g}$$

Appendix 80

Variation of yeast yield with no polymer present

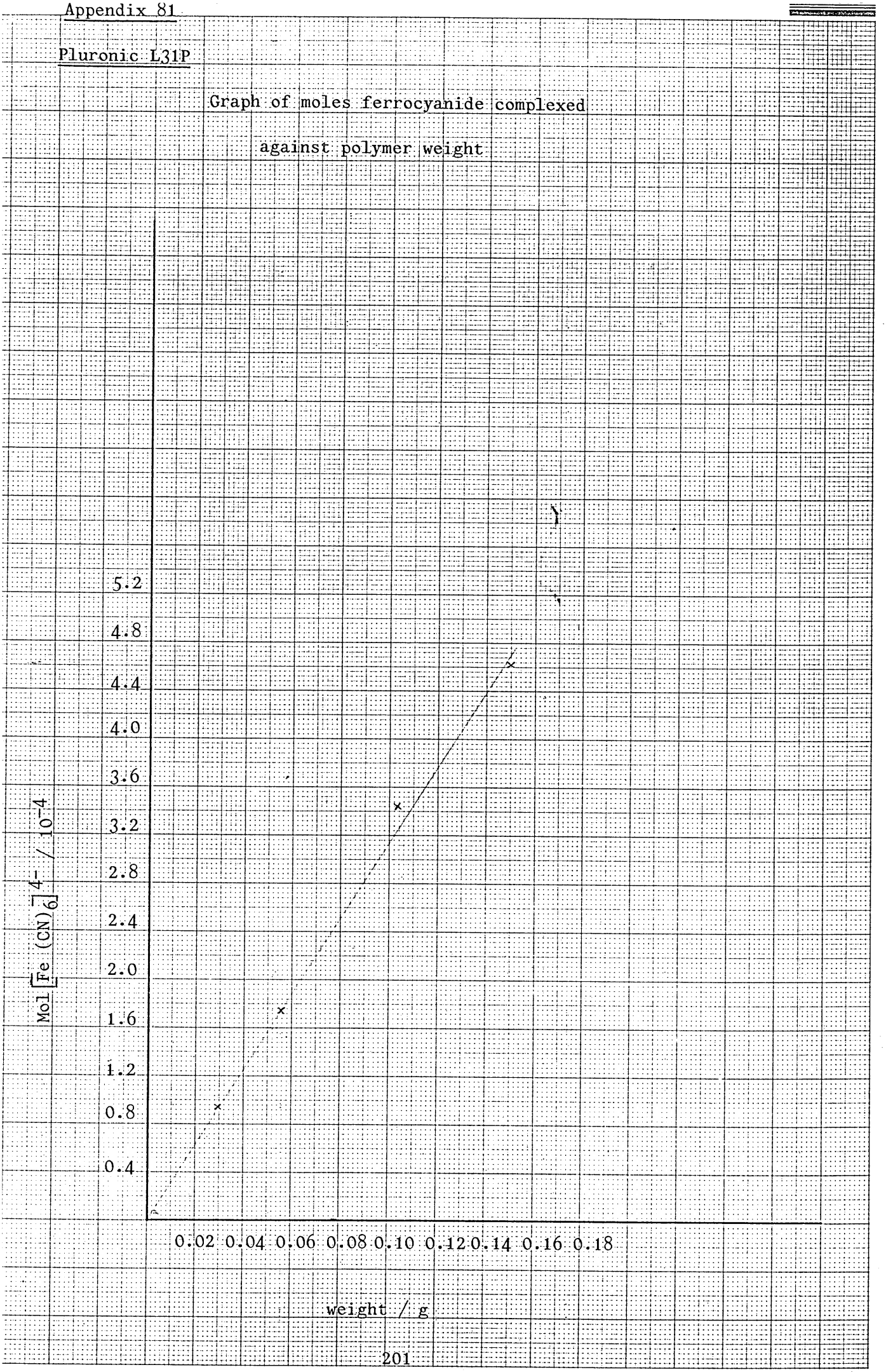
Yeast yield = 2.388 g

σ_{n-1} = 11.10×10^{-3} g

σ_n = 9.06×10^{-3} g

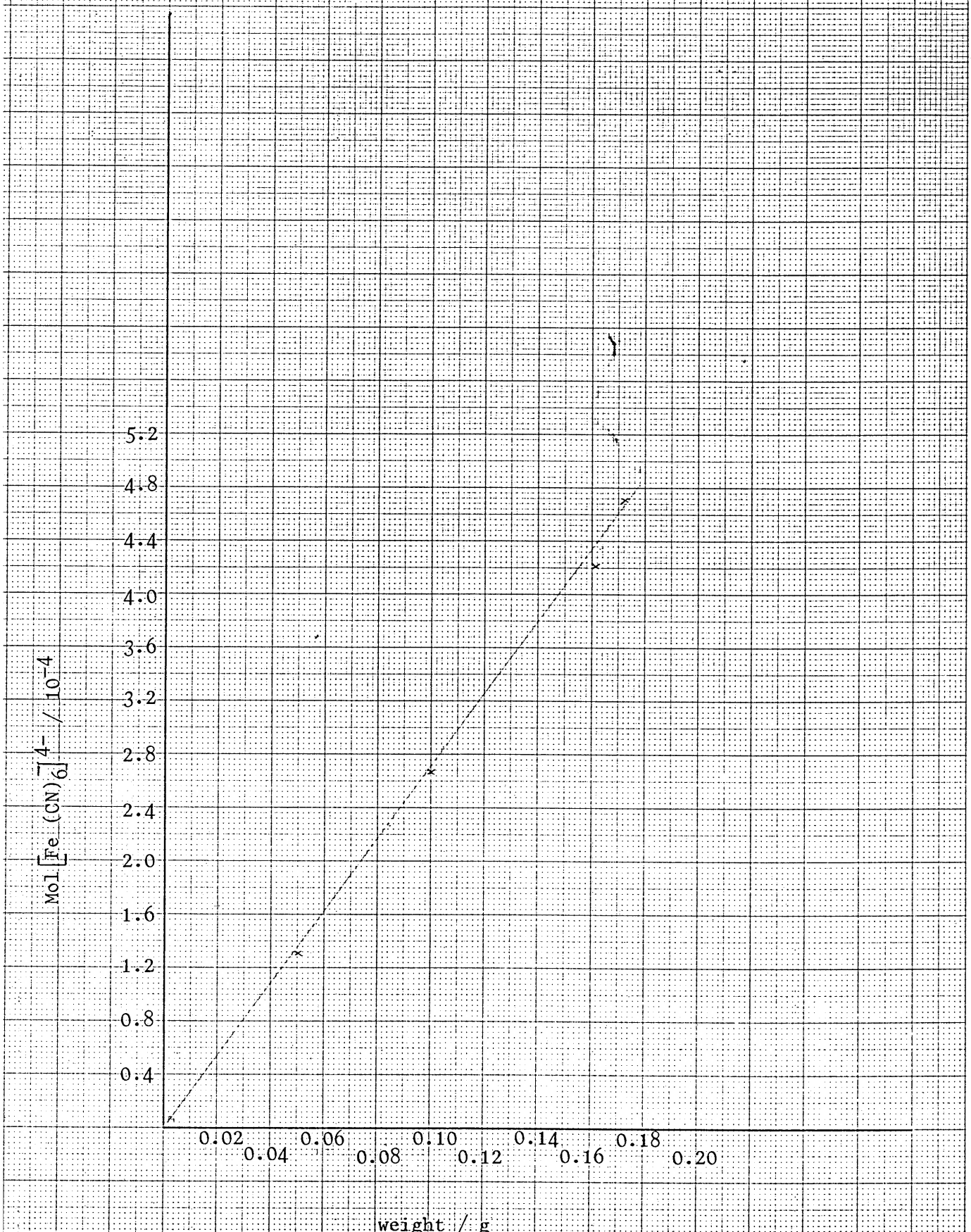
Pluronic L31P

Graph of moles ferrocyanide complexed against polymer weight



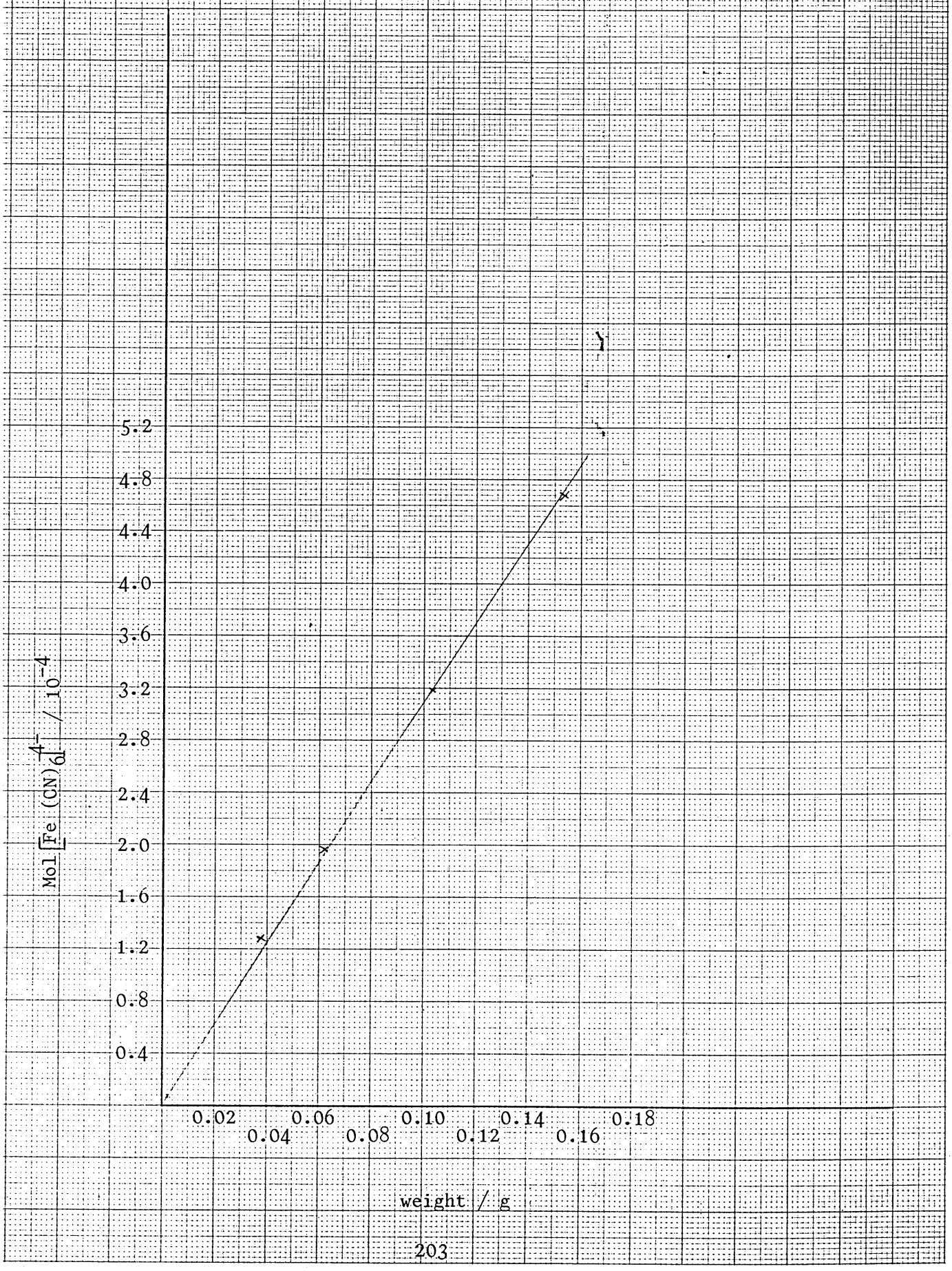
Pluronic L61P

Graph of moles ferrocyanide complexed against polymer weight



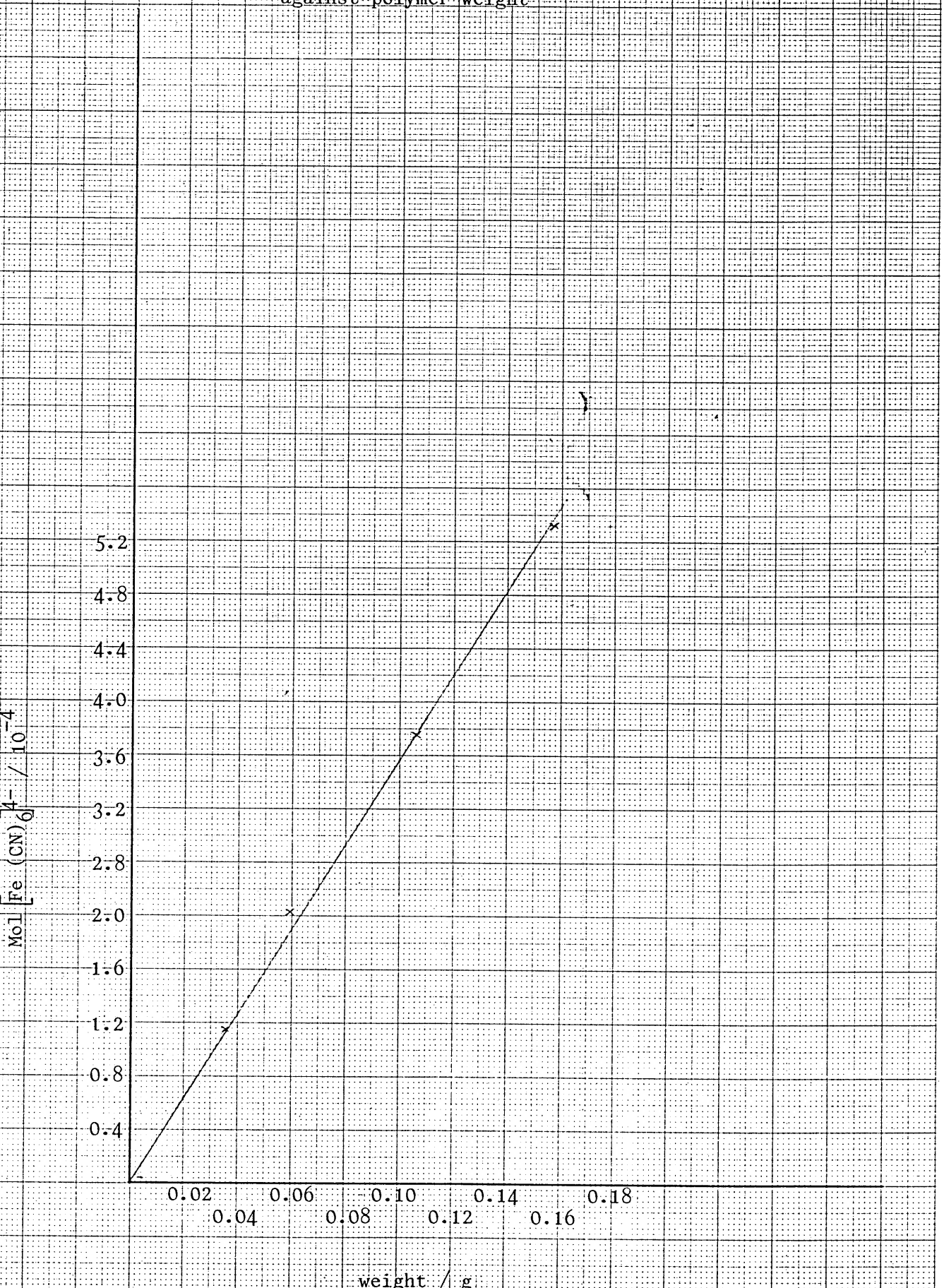
Pluronic L62P

Graph of moles ferrocyanide complexed against polymer weight



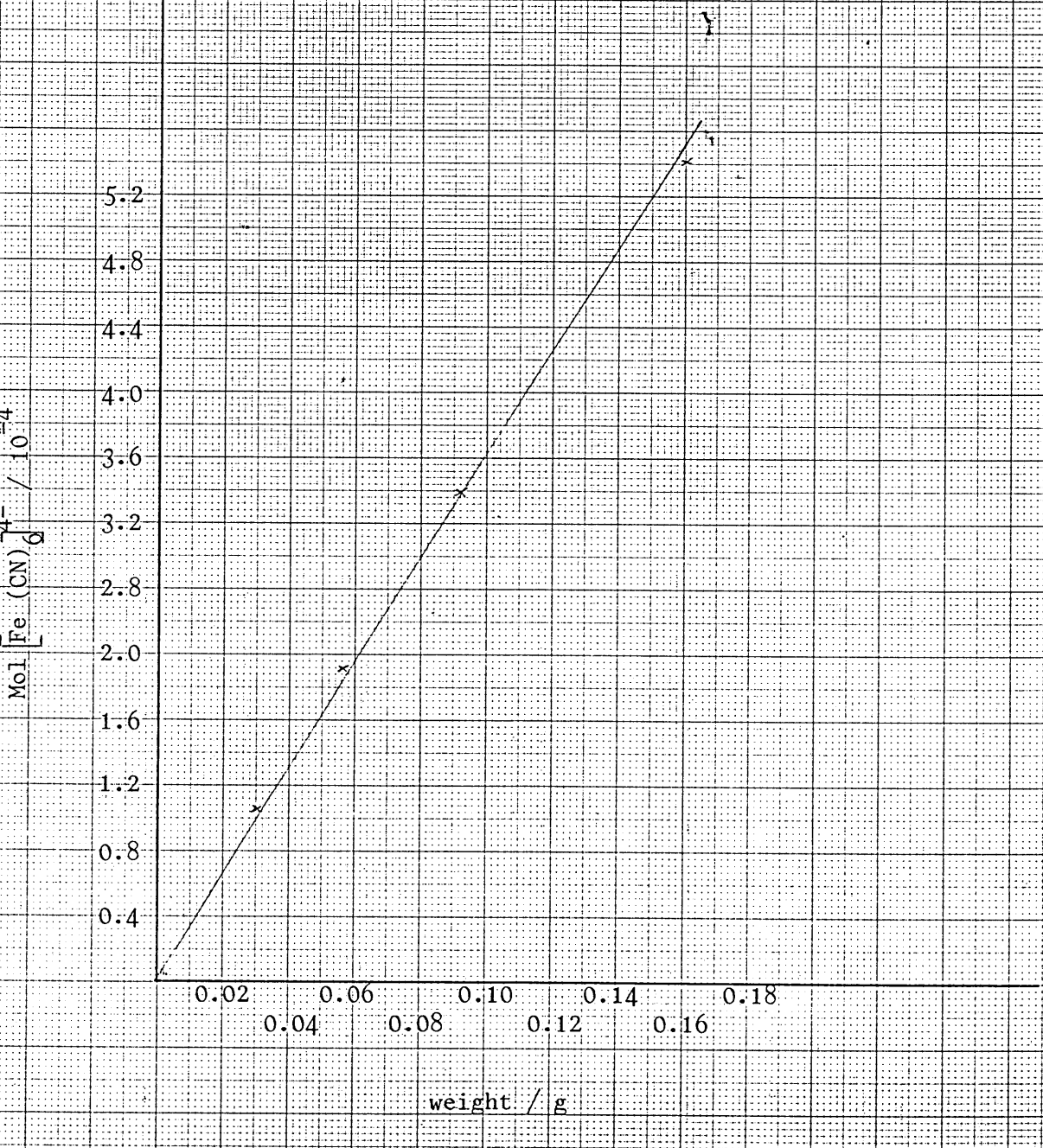
Pluronic L63P

Graph of moles ferrocyanide complexed against polymer weight



Pluronic L64P

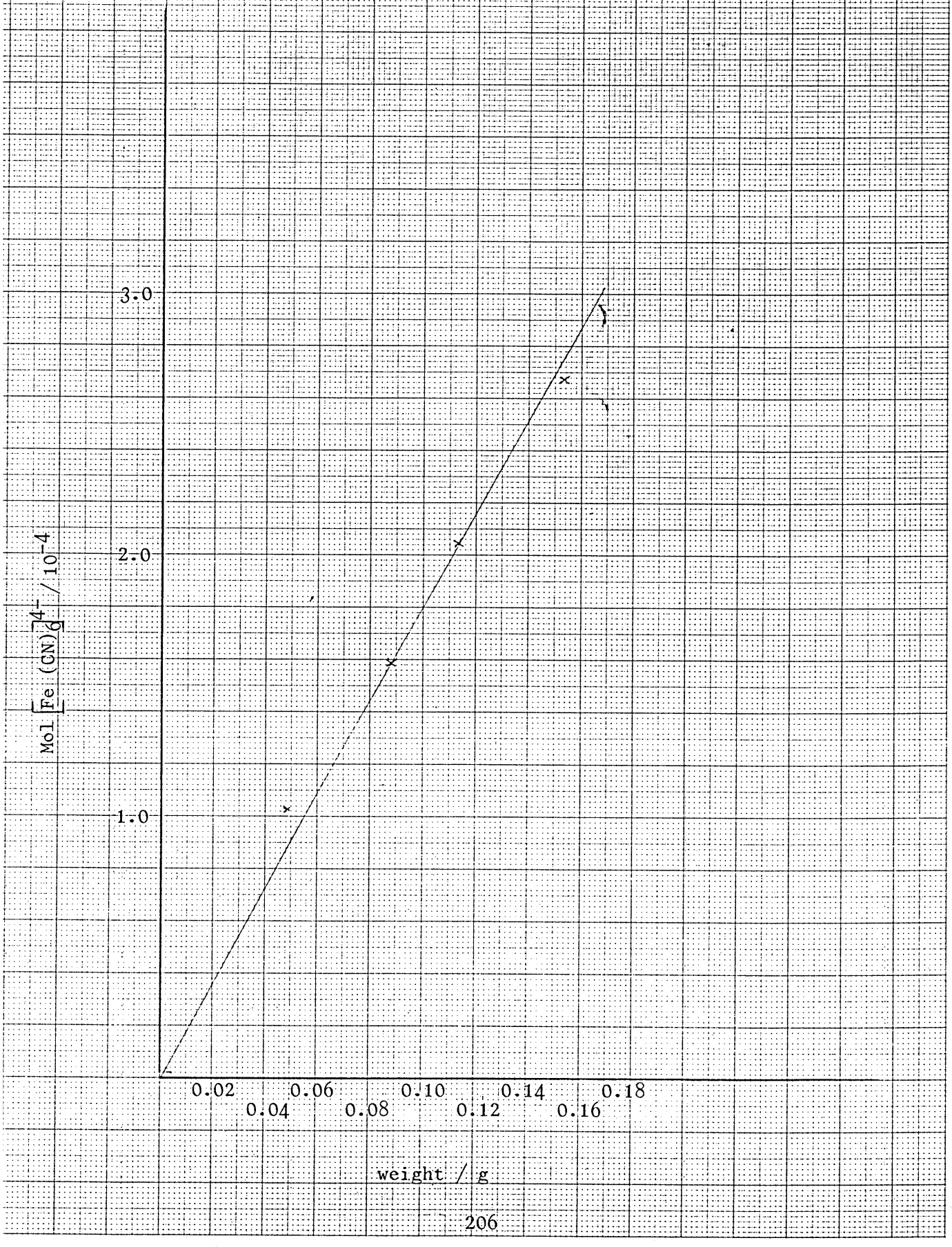
Graph of moles ferrocyanide complexed against polymer weight



Appendix 80

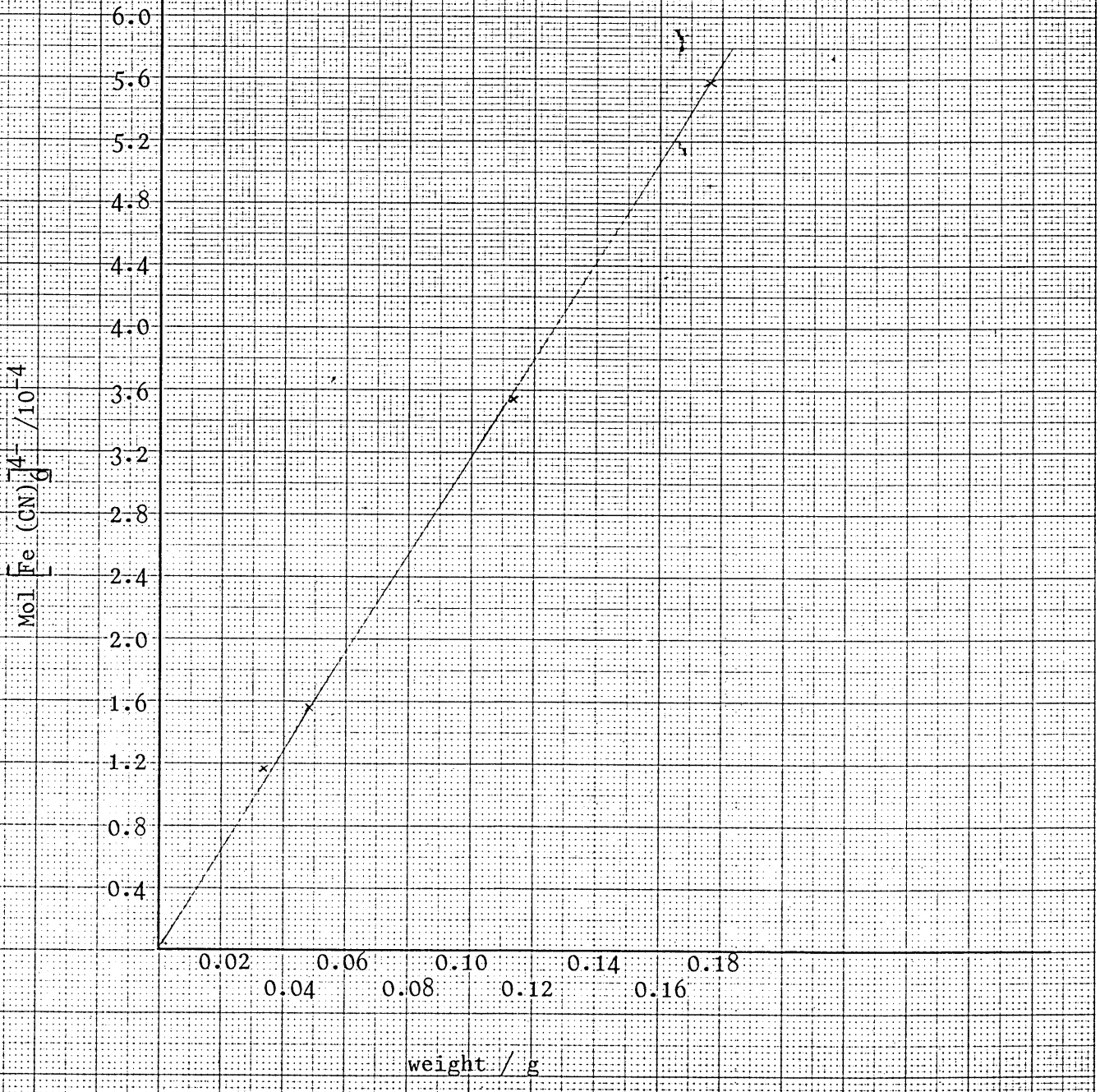
Pluronic L81P

Graph of moles ferrocyanide complexed against polymer weight



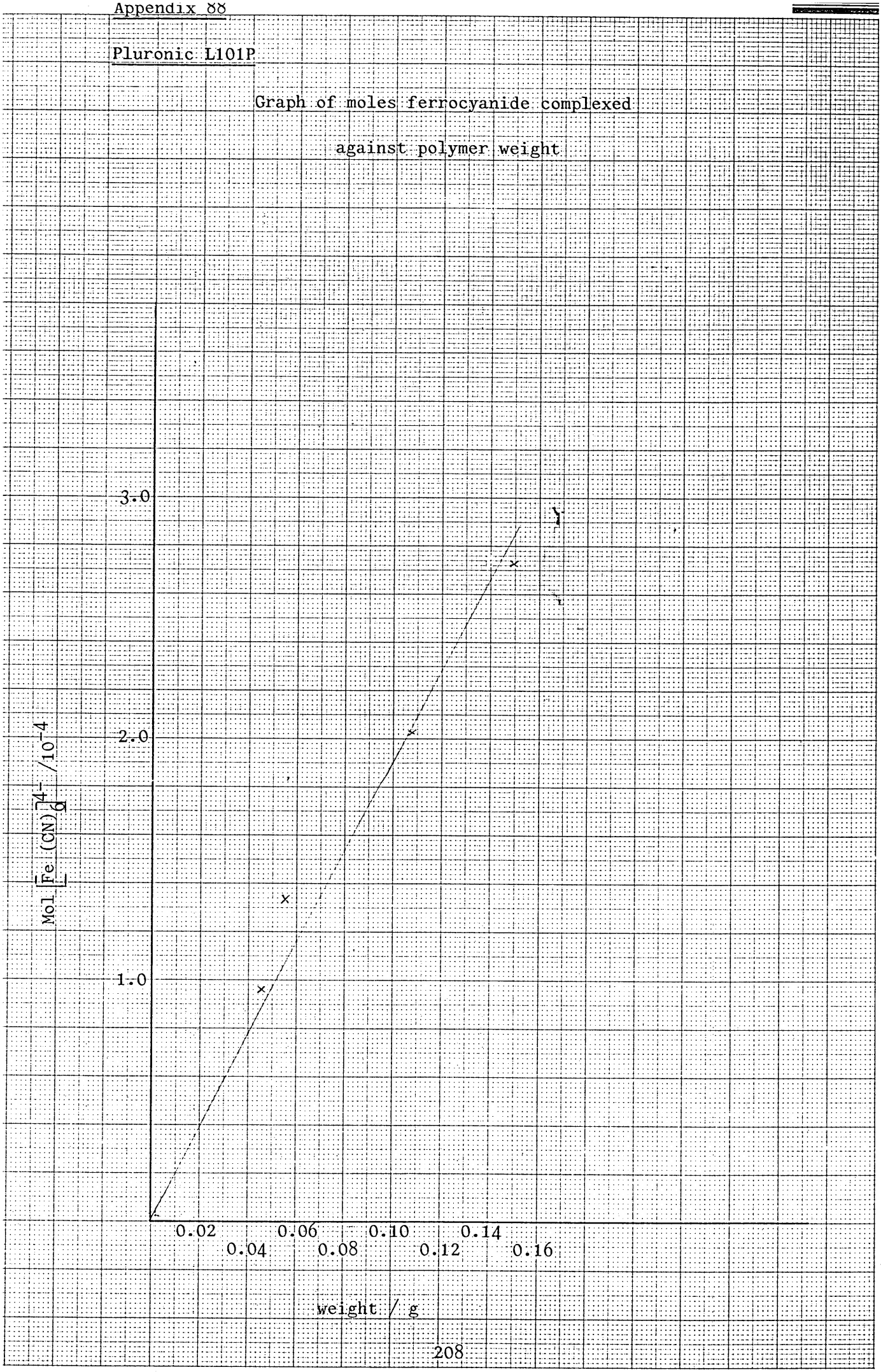
Pluronic L92P

Graph of moles ferrocyanide complexed against polymer weight



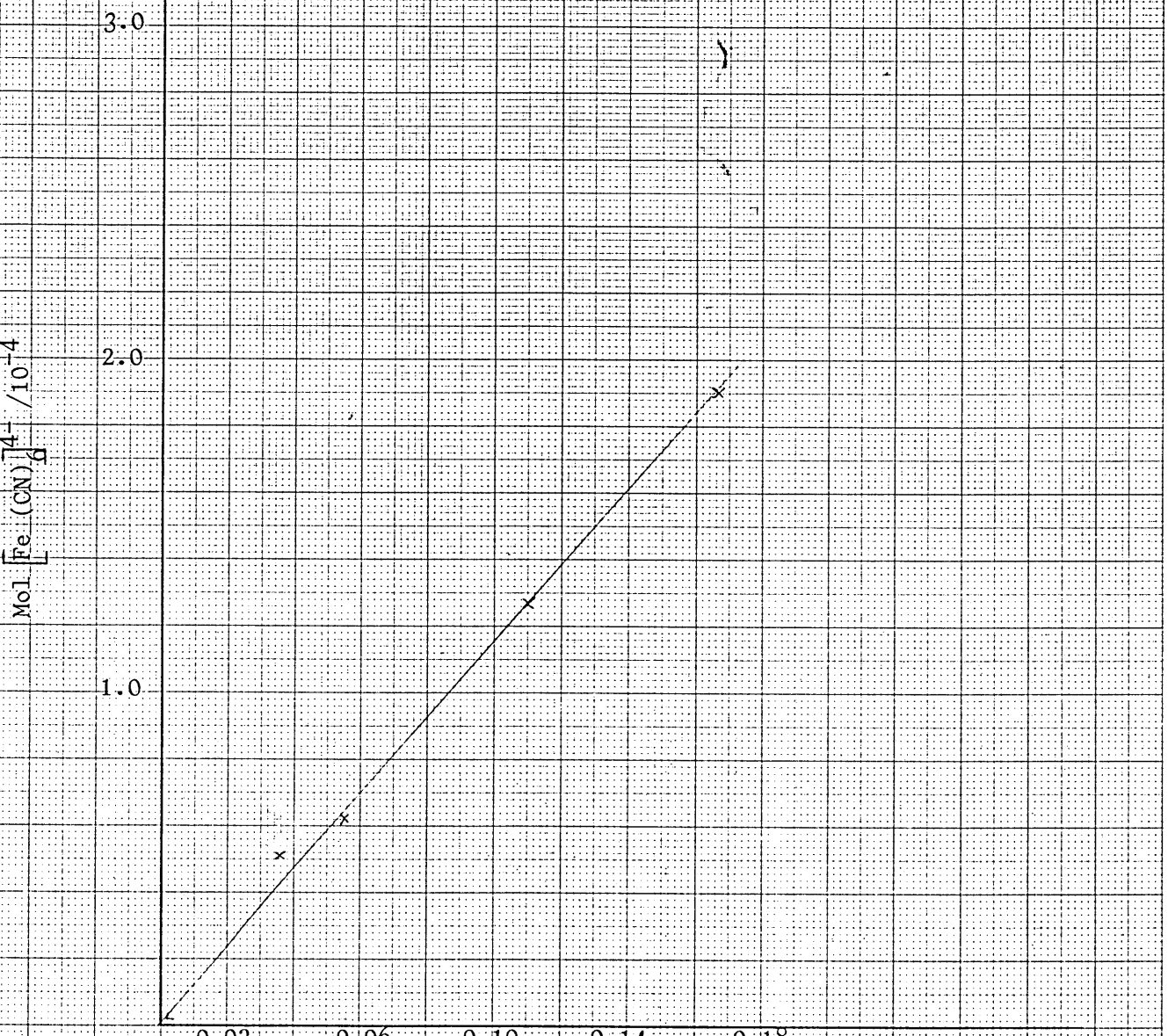
Pluronic L101P

Graph of moles ferrocyanide complexed against polymer weight



Pluronic L121P

Graph of moles ferrocyanide complexed against polymer weight



Appendix 90

Variation of moles of ethylene oxide and propylene oxide with moles of ferrocyanide complexed

Pluronic L31P

POLYMER WEIGHT / g	Mw	A MOLES ETHYLENE OXIDE / 10^{-4}	B MOLES PROPYLENE OXIDE / 10^{-4}	C MOLES POLYMER / 10^{-4}	D MOLES $[Fe(CN)_6]^{4-}$ COMPLEXED/10^{-4}	D/A	D/B	D/C
0.029	974	0.718	4.055	0.298	0.945	1.316	0.233	3.171
0.053	974	1.313	7.411	0.544	1.769	1.347	0.238	3.252
0.103	974	2.552	14.403	1.057	3.319	1.301	0.230	3.140
0.150	974	3.716	20.974	1.540	4.625	1.245	0.221	3.003
					MEAN	1.302	0.231	3.142
					σ_{n-1}	0.033	0.031	0.033
					σ_n	0.028	0.027	0.029

Appendix 91

Variation of moles of ethylene oxide and propylene oxide with moles of ferrocyanide complexed

Pluronic L61P

POLYMER WEIGHT / g	Mw	A MOLES ETHYLENE OXIDE / 10^{-4}	B MOLES PROPYLENE OXIDE / 10^{-4}	C MOLES POLYMER / 10^{-4}	D MOLES $[Fe(CN)_6]^{4-}$ COMPLEXED/10^{-4}	D/A	D/B	D/C
0.100	2271	2.480	15.223	0.440	2.675	1.079	0.176	6.080
0.111	2271	2.753	16.898	0.489	3.286	1.194	0.194	6.720
0.161	2271	3.993	24.510	0.709	4.207	1.054	0.172	6.226
0.172	2271	4.266	26.184	0.757	4.713	1.105	0.180	6.226
					MEAN	1.108	0.181	6.313
					σ_{n-1}	0.055	0.053	0.044
					σ_n	0.048	0.046	0.038

Appendix 92

Variation of moles of ethylene oxide and propylene oxide with moles of ferrocyanide complexed

Pluronic L62P

POLYMER WEIGHT / g	Mw	A MOLES ETHYLENE OXIDE / 10^{-4}	B MOLES PROPYLENE OXIDE / 10^{-4}	C MOLES POLYMER / 10^{-4}	D MOLES $[Fe(CN)_6]^{4-}$ COMPLEXED/10^{-4}	D/A	D/B	D/C
0.038	2363	2.014	4.974	0.161	1.291	0.641	0.260	8.019
0.061	2363	3.233	7.985	0.258	1.968	0.609	0.246	7.628
0.103	2363	5.459	13.483	0.436	3.165	0.580	0.235	7.259
0.152	2363	8.056	19.897	0.643	4.678	0.581	0.235	7.275
					MEAN	0.603	0.244	7.545
					σ_{n-1}	0.048	0.051	0.048
					σ_n	0.041	0.044	0.041

Appendix 93

Variation of moles of ethylene oxide and propylene oxide with moles of ferrocyanide complexed

Pluronic L63P

POLYMER WEIGHT / g	Mw	A MOLES ETHYLENE OXIDE / 10^{-4}	B MOLES PROPYLENE OXIDE / 10^{-4}	C MOLES POLYMER / 10^{-4}	D MOLES $[Fe(CN)_6]^{4-}$ COMPLEXED/10^{-4}	D/A	D/B	D/C
0.035	2273	2.060	4.424	0.154	1.149	0.558	0.260	7.461
0.059	2273	3.473	7.458	0.260	2.040	0.587	0.274	7.846
0.106	2273	6.239	13.398	0.466	3.343	0.536	0.250	7.174
0.157	2273	9.241	19.845	0.691	4.913	0.532	0.248	7.110
					MEAN	0.553	0.258	7.398
					σ_{n-1}	0.046	0.046	0.045
					σ_n	0.040	0.040	0.039

Appendix 94

Variation of moles of ethylene oxide and propylene oxide with moles of ferrocyanide complexed

Pluronic L64P

POLYMER WEIGHT / g	Mw	A MOLES ETHYLENE OXIDE / 10^{-4}	B MOLES PROPYLENE OXIDE / 10^{-4}	C MOLES POLYMER / 10^{-4}	D MOLES $[Fe(CN)_6]^{4-}$ COMPLEXED/10^{-4}	D/A	D/B	D/C
0.030	2477	2.633	3.137	0.121	1.056	0.401	0.337	8.727
0.056	2477	4.915	5.856	0.226	1.922	0.391	0.328	8.504
0.092	2477	8.075	9.620	0.371	2.984	0.370	0.310	8.043
0.160	2477	14.043	16.730	0.646	5.009	0.357	0.299	7.754
					MEAN	0.380	0.319	8.257
					σ_{n-1}	0.052	0.054	0.053
					σ_n	0.045	0.047	0.046

Appendix 95

Variation of moles of ethylene oxide and propylene oxide with moles of ferrocyanide complexed

Pluronic L81P

POLYMER WEIGHT / g	Mw	A MOLES ETHYLENE OXIDE / 10^{-4}	B MOLES PROPYLENE OXIDE / 10^{-4}	C MOLES POLYMER / 10^{-4}	D MOLES $[Fe(CN)_6]^{4-}$ COMPLEXED/10^{-4}	D/A	D/B	D/C
0.048	2814	1.583	7.022	0.171	1.028	0.649	0.146	6.012
0.088	2814	2.902	12.874	0.313	1.598	0.551	0.124	5.105
0.112	2814	3.694	16.385	0.398	2.054	0.556	0.125	5.161
0.152	2814	5.013	22.237	0.540	2.674	0.533	0.120	4.952
					MEAN	0.572	0.129	5.308
					σ_{n-1}	0.091	0.090	0.090
					σ_n	0.079	0.078	0.078

Appendix 96

Variation of moles of ethylene oxide and propylene oxide with moles of ferrocyanide complexed

Pluronic L92P

POLYMER WEIGHT / g	Mw	A MOLES ETHYLENE OXIDE / 10^{-4}	B MOLES PROPYLENE OXIDE / 10^{-4}	C MOLES POLYMER / 10^{-4}	D MOLES $[Fe(CN)_6]^{4-}$ COMPLEXED/10^{-4}	D/A	D/B	D/C
0.033	3427	1.582	4.482	0.096	1.171	0.740	0.261	12.198
0.048	3427	2.301	6.519	0.140	1.564	0.680	0.240	11.171
0.113	3427	5.417	15.347	0.330	3.556	0.656	0.232	10.776
0.176	3427	8.437	23.903	0.514	5.589	0.662	0.234	10.874
					MEAN	0.684	0.242	11.255
					σ_{n-1}	0.056	0.055	0.058
					σ_n	0.049	0.048	0.050

Appendix 97

Variation of moles of ethylene oxide and propylene oxide with moles of ferrocyanide complexed

Pluronic L101P

POLYMER WEIGHT / g	Mw	A MOLES ETHYLENE OXIDE / 10^{-4}	B MOLES PROPYLENE OXIDE / 10^{-4}	C MOLES POLYMER / 10^{-4}	D MOLES $[Fe(CN)_6]^{4-}$ COMPLEXED/10^{-4}	D/A	D/B	D/C
0.042	3893	1.226	6.278	0.108	0.961	0.784	0.153	8.898
0.055	3893	1.605	8.221	0.143	1.354	0.844	0.165	9.469
0.107	3893	3.123	15.994	0.275	2.017	0.646	0.126	7.335
0.139	3893	4.057	20.777	0.357	2.725	0.672	0.131	7.633
					MEAN	0.737	0.144	8.334
					σ_{n-1}	0.127	0.128	0.122
					σ_n	0.110	0.111	0.106

Appendix 98

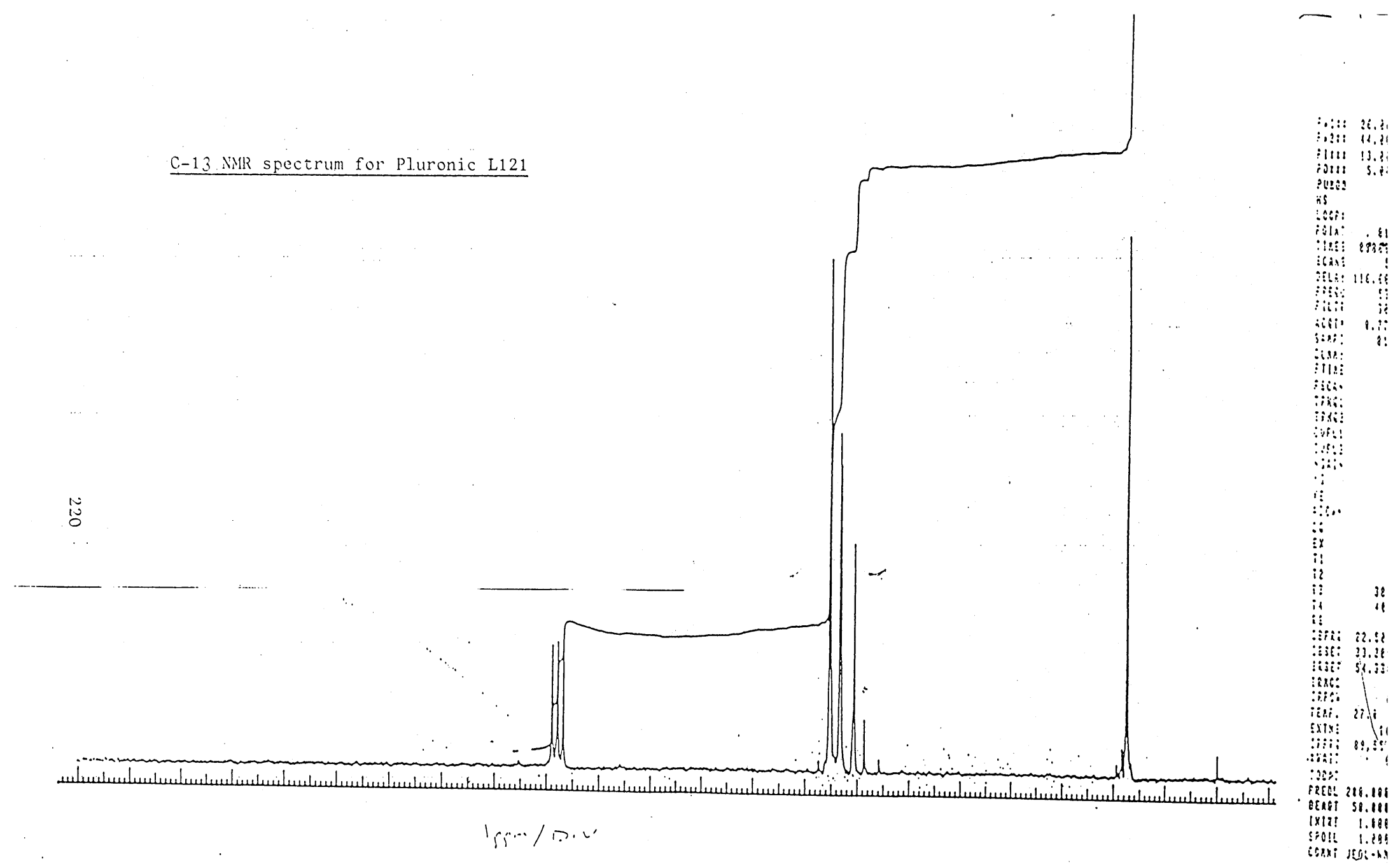
Variation of moles of ethylene oxide and propylene oxide with moles of ferrocyanide complexed

Pluronic L121P

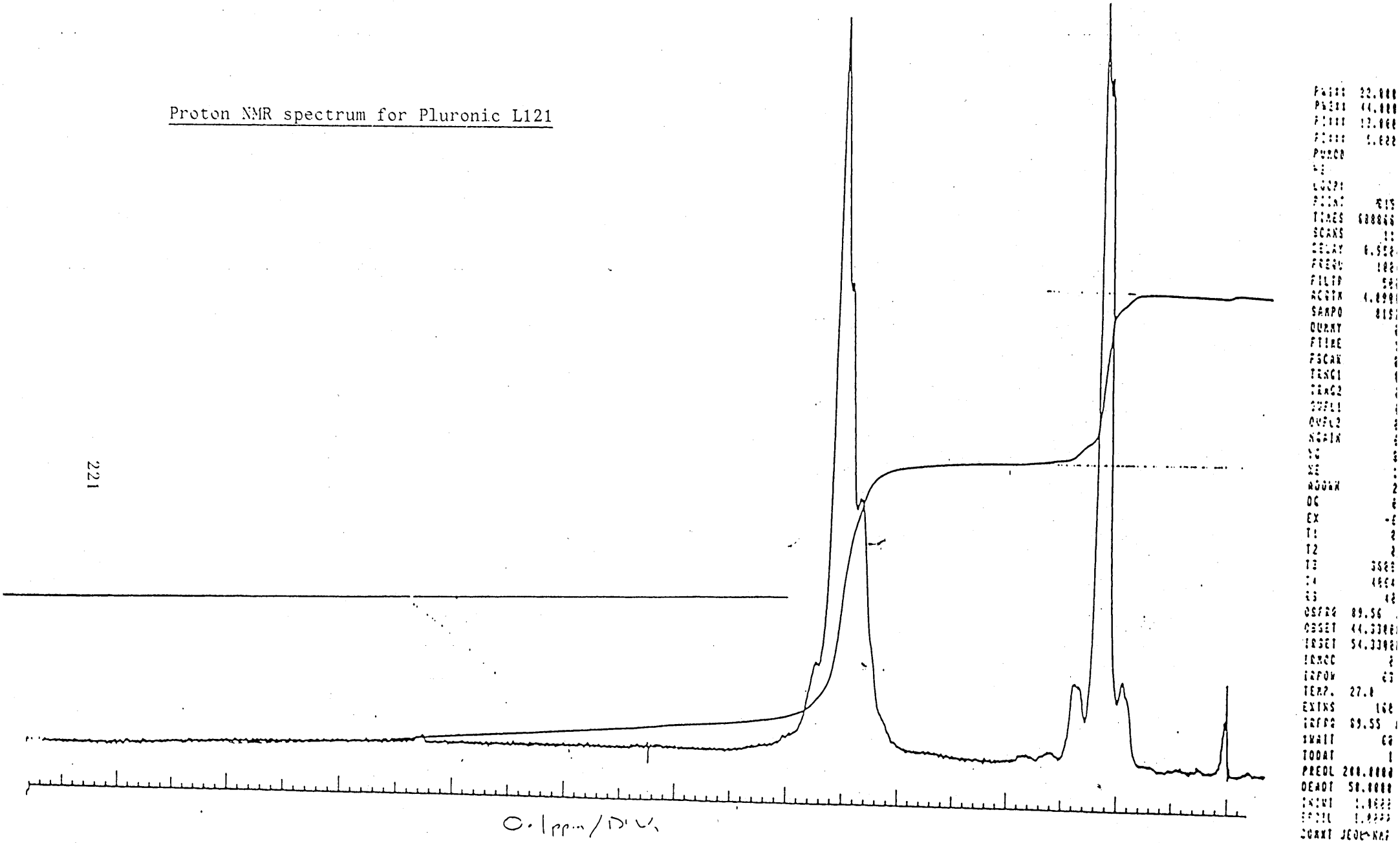
POLYMER WEIGHT / g	Mw	A MOLES ETHYLENE OXIDE / 10^{-4}	B MOLES PROPYLENE OXIDE / 10^{-4}	C MOLES POLYMER / 10^{-4}	D MOLES $[Fe(CN)_6]^{4-}$ COMPLEXED/10^{-4}	D/A	D/B	D/C
0.035	4027	0.998	4.650	0.087	0.515	0.516	0.111	5.920
0.055	4027	1.568	7.307	0.137	0.620	0.395	0.085	5.526
0.110	4027	3.137	14.614	0.273	1.271	0.405	0.087	4.656
0.166	4027	4.733	22.054	0.412	1.889	0.399	0.086	4.585
					MEAN	0.429	0.092	5.172
					σ_{n-1}	0.136	0.136	0.127
					σ_n	0.118	0.118	0.110

Appendix 99 ^{1}H and ^{13}C NMR spectra for typical ethylene oxide-propylene oxide copolymer

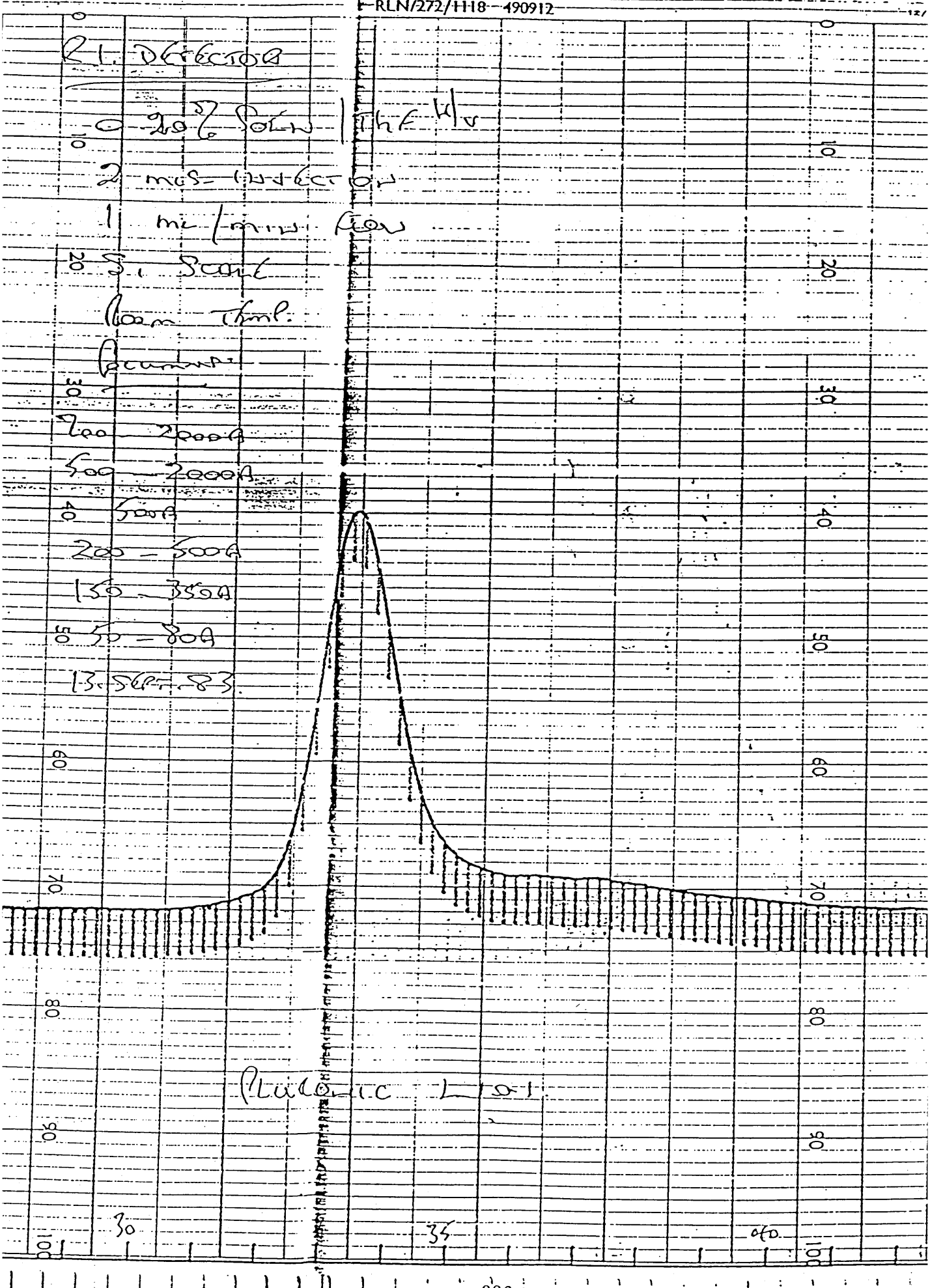
C-13 NMR spectrum for Pluronic L121



Proton NMR spectrum for Pluronic L121



Appendix 100 GPC chromatogram for typical ethylene oxide - propylene oxide copolymer


RI Detector
0-20 mV FSDN
2 mV RI detector
1 ml/min flow
RI Scale
Room Temp.
Accumap
400 - 2000 A
500 - 2000 A
500 A
200 - 500 A
150 - 350 A
50 - 80 A
13-Sept-83
Fluoro-1.C-1-01
30
35
40
223

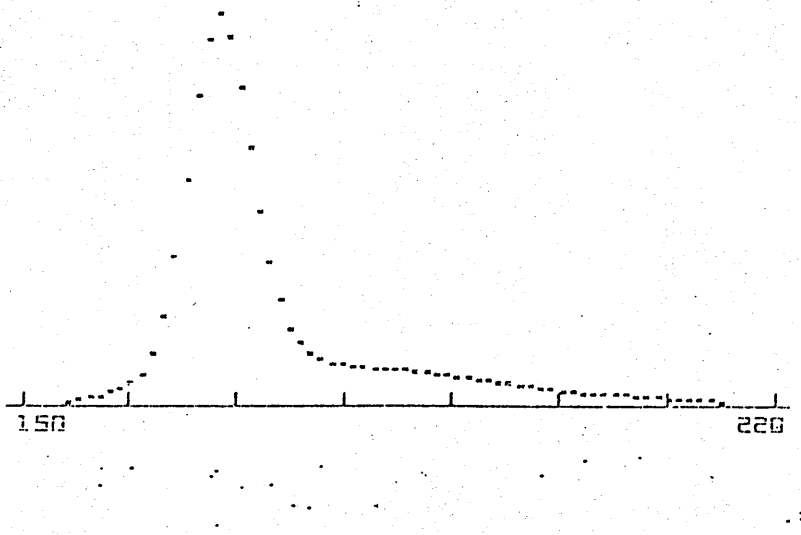
CHROMATOGRAM

SAMPLE : PLURONIC L101
DATE OF ANALYSIS : 14 9 83
SOLVENT : THF
COLUMN SET : LOW

CONCENTRATION : .2
SENSITIVITY : 8

INITIAL VOLUME : 154
VOLUME INCREMENT : .9435
NUMBER OF DATA POINTS : 63

UNCORRECTED DATA



AREA UNDER CHROMATOGRAM = 1333.7

REDUCED AREA ,A/(CONC*SENS), = 833.6

MEAN ELUTION VOLUME (Vw) = 170.8

CHAPTER 8 - References

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